Impaired Responsiveness to NO in Newly Diagnosed Patients With Rheumatoid Arthritis

Robert Bergholm, Marjatta Leirisalo-Repo, Satu Vehkavaara, Sari Mäkimattila, Marja-Riitta Taskinen, Hannele Yki-Järvinen

Objective—Cardiovascular disease is the major cause of excessive mortality in patients with rheumatoid arthritis (RA). We determined whether endothelial dysfunction characterizes patients with newly diagnosed RA (n = 10) compared with normal subjects (control group, n = 33) and whether it is reversible with 6 months of anti-inflammatory therapy.

Methods and Results—Endothelial function was determined by measuring vasodilatory responses to intrabrachial artery infusions of acetylcholine (ACh at 7.5 and 15 μg/min, low and high dose, respectively), an endothelium-dependent vasodilator, and to sodium nitroprusside (SNP, 3 and 10 μg/min), an endothelium-independent vasodilator. Before treatment, blood flow responses (fold increase in flow) to low-dose SNP were 30% lower in the RA versus the control group (4.1 ± 0.4-fold versus 5.9 ± 0.5-fold, respectively), and responses to high-dose SNP were 34% lower in the RA group versus the control group (5.1 ± 0.6-fold versus 7.7 ± 0.7-fold, respectively; P < 0.001). The responses to low-dose ACh were 50% lower in the RA group versus the control group (3.0 ± 0.5-fold versus 6.6 ± 0.7-fold, respectively), and responses to high-dose ACh were 37% lower in the RA group versus the control group (4.1 ± 0.4-fold versus 5.9 ± 0.8-fold, respectively; P < 0.001). After therapy, clinical and laboratory markers of inflammation had significantly decreased. Blood flow responses to ACh increased significantly (P = 0.02).

Conclusions—We conclude that newly diagnosed patients with RA have vascular dysfunction, which is reversible with successful therapy. Therefore, early suppression of inflammatory activity may reduce long-term vascular damage. (Arterioscler Thromb Vasc Biol. 2002;22:1637-1641.)

Key Words: atherosclerosis □ blood vessels □ vasodilatation

In recent years, coronary artery disease has been recognized as the major cause of excess morbidity and mortality in patients with rheumatoid arthritis (RA).1-5 Because of parallels between inflammatory/autoimmune diseases and atherosclerosis, it has been suggested that various inflammatory mediators may contribute to vascular dysfunction in patients with RA.6 The similarities include increases in circulating concentrations of adhesion molecules, proinflammatory cytokines, and acute-phase proteins in patients with RA as well as in subjects with cardiovascular risk factors or overt cardiovascular disease.6-8 In humans, local administration of tumor necrosis factor (TNF)-α and interleukin-1β increases basal NO-dependent venodilatation but impairs endothelium-dependent venodilatation induced by bradykinin.9 These experimental data suggest that inflammatory disorders could predispose an individual to cardiovascular disease via blunting of endothelial- and NO-dependent vasodilatation. However, the pathophysiological relevance of these data is uncertain, because it is unknown whether in vivo endothelial dysfunction indeed characterizes chronic human inflammatory diseases such as RA. In the present study, we determined whether blood flow responses to intra-arterial endothelium-dependent and -independent vasodilators are altered in untreated patients with newly diagnosed RA and, if so, whether vascular dysfunction can be ameliorated by anti-inflammatory therapy.

Methods

Subjects
Ten patients with early RA (duration of symptoms ≤18 months) were studied before and 6 months after the initiation of therapy. Before the first vascular function study, no patient had received treatment with disease-modifying antirheumatic drugs (DMARDs) or oral prednisone. A total of 33 matched normal subjects were studied as a control group. Clinical and biochemical characteristics of the study groups are shown in Table 1. The patients fulfilled the 1987 American College of Rheumatology criteria for RA.10 Eight of the patients were rheumatoid factor positive. None of the patients had detectable levels of antinuclear or anticentromere antibodies. One of the patients had rheumatoid vasculitis and rheumatoid nodules. No other patient had extra-articular symptoms or signs of secondary Sjögren’s syndrome. Two of the patients had erosions at the time of diagnosis. None of the patients or normal subjects had hypertension or a history of cardiovascular disease, and none of the...
Design

Vascular function was measured after a 10- to 12-hour overnight fast, as detailed below. Before arterial cannulation, an intravenous catheter was inserted into the right medial antecubital vein to withdraw blood samples for measurement of concentrations of serum lipids and lipoproteins and C-reactive protein, TNF-α, and the erythrocyte sedimentation rate. The purpose, nature, and potential risks of the studies were explained to the patients before their written informed consent was obtained. The experimental protocol was approved by the ethics committee of the Helsinki University Hospital.

Assessment of Endothelium-Dependent and -Independent Vasodilatation

Vascular function was assessed in forearm resistance vessels by measuring forearm blood flow responses to intra-arterial infusions of endothelium-dependent (ACh) and -independent (SNP) vasodilators, as previously described in detail. A 27-gauge unmounted steel cannula (Coopers Needle Works), connected to an epidural catheter (Portex), was inserted into the left brachial artery. All drugs were infused at a constant rate of 1 mL/min with infusion pumps (B. Braun AG). The subjects rested supine in a quiet environment for 30 minutes after needle placement before blood flow measurements were begun. Normal saline was first infused for 18 minutes. Drugs were then infused in the following sequence: SNP (Nitropress, Abbott Laboratories) at 3 μg/min (low dose) and 10 μg/min (high dose) and ACh (Miochol, OMJ Pharmaceuticals) at 7.5 μg/min (low dose) and 15 μg/min (high dose). These doses were chosen because they induce comparable increases in forearm blood flow in normal subjects. Each dose was infused for 6 minutes, and the infusion of each drug was separated by the infusion of normal saline for 18 minutes, during which blood flow returned to basal values. Forearm blood flow was recorded for 10 seconds at 15-second intervals during the last 3 minutes of each drug and saline infusion period with the use of a mercury-in-rubber strain-gauge venous occlusion plethysmograph (EC 4 Strain Gauge Plethysmograph, Hokanson), which was connected to a rapid cuff inflator (E 20, Hokanson), an analog-to-digital converter (McLab/4e, AD Instruments), and a personal computer, as previously described. Blood flow measurements were performed simultaneously in the infused (experimental) and control arm. Means of the final 5 measurements of each recording period were used for analysis. Blood pressure and heart rate were measured before and after the endothelial function test. Blood flow results during infusion of vasodilators SNP and ACh are reported as a ratio of blood flow in the experimental arm divided by the blood flow in the control arm to correct for any differences in basal flow.

TABLE 1. Clinical and Biochemical Characteristics of the Study Groups

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Newly Diagnosed</th>
<th>Before Treatment (n=10)</th>
<th>After Treatment (n=10)</th>
<th>Normal Subjects (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, women/men</td>
<td>8/2</td>
<td>27/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>52±3</td>
<td>54±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74±4</td>
<td>74±2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27±1</td>
<td>27±1</td>
<td>26±1</td>
<td></td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.87±0.02</td>
<td>0.87±0.02</td>
<td>0.86±0.02</td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>133±7</td>
<td>132±4</td>
<td>135±4</td>
<td></td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>76±3*</td>
<td>77±2</td>
<td>81±2</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>128±5**</td>
<td>128±3†††</td>
<td>140±1</td>
<td></td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>40±7***</td>
<td>19±2††††‡</td>
<td>8±1</td>
<td></td>
</tr>
<tr>
<td>S-CRP, mg/L</td>
<td>29±10***</td>
<td>8±3††††‡</td>
<td>4±1</td>
<td></td>
</tr>
<tr>
<td>S-TNF-α, ng/L</td>
<td>3±0.5***</td>
<td>2.3±0.5†††‡</td>
<td>1.5±0.2</td>
<td></td>
</tr>
<tr>
<td>No. of tender joints</td>
<td>11±2</td>
<td>4±1†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of swollen joints</td>
<td>8±3</td>
<td>4±2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain (pain scale 0–10 cm)</td>
<td>4.4±0.8</td>
<td>2.3±1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>3/10</td>
<td>11/33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as mean±SEM. CRP indicates C-reactive protein; ESR, erythrocyte sedimentation rate; TNF-α, tumor necrosis factor α; SBP, systolic blood pressure; DBP, diastolic blood pressure. *P<0.05, **P<0.01, ***P<0.001 for RA patients before treatment vs normal subjects. ††P<0.05, †††P<0.01, ††††P<0.001 for RA patients after treatment vs normal subjects. †††P<0.05 for RA before vs after treatment.

normal subjects used any drugs. After the basal study, the patients were started with DMARDs if they had clinically active disease. Nonsteroidal anti-inflammatory drugs were prescribed as symptom-therapy. Treatment of the RA patients consisted of methotrexate in 5 patients and cyclophosphamide in 1 patient with rheumatic vasculitis. Four patients were on low-dose (5 to 7.5 mg) prednisone, and 7 used nonsteroidal anti-inflammatory drugs. Before the endothelial function test, the patients were instructed not to take acetylsalicylic acid or other nonsteroidal anti-inflammatory drugs for 24 hours (low dose) and ACh (Miochol, OMJ Pharmaceuticals) at 7.5 μg/min (low dose) and 15 μg/min (high dose). These doses were chosen because they induce comparable increases in forearm blood flow in normal subjects. Each dose was infused for 6 minutes, and the infusion of each drug was separated by the infusion of normal saline for 18 minutes, during which blood flow returned to basal values. Forearm blood flow was recorded for 10 seconds at 15-second intervals during the last 3 minutes of each drug and saline infusion period with the use of a mercury-in-rubber strain-gauge venous occlusion plethysmograph (EC 4 Strain Gauge Plethysmograph, Hokanson), which was connected to a rapid cuff inflator (E 20, Hokanson), an analog-to-digital converter (McLab/4e, AD Instruments), and a personal computer, as previously described. Blood flow measurements were performed simultaneously in the infused (experimental) and control arm. Means of the final 5 measurements of each recording period were used for analysis. Blood pressure and heart rate were measured before and after the endothelial function test. Blood flow results during infusion of vasodilators SNP and ACh are reported as a ratio of blood flow in the experimental arm divided by the blood flow in the control arm to correct for any differences in basal flow.

Serum Lipids, Lipoproteins, and Apoproteins

Serum lipoproteins were isolated by ultracentrifugation as previously described. The concentrations of cholesterol and triglycerides in serum and lipoprotein subfractions were determined by enzymatic colorimetric assays (Hoffman-La Roche) with the use of an autoanalyzer (Cobas Mira, Hoffman-La Roche). The concentrations of serum apoA-I, apoA-II, and apoB were determined by using commercially available immunoturbidimetric assays (Boehringer-Mannheim for apoA-I and apoA-II and Orion Diagnostica for apoB).

Other Measurements

Serum TNF-α concentrations were measured by using a high-sensitivity ELISA kit from R&D Systems. The percentage of whole body fat was measured by a single-frequency bioelectrical impedance device (model BIA-101A, Bio-Electrical Impedance Analyzer System).

Statistical Analyses

The Student unpaired t test was used to compare single measurements between the patients with RA and the normal subjects. Single measurements before and after therapy were compared by using the Student paired t test. Comparison of blood flow responses to the 2 doses of vasoactive drugs was performed by ANOVA for repeated measures. Correlation analyses were calculated by using the Spearman nonparametric rank correlation coefficient. A value of P<0.05 was considered statistically significant. The calculations were performed by using the GraphPad Prism, version 2.01, statistical program or Systat, version 10 (SPSS). All data are shown as mean±SEM.

Results

RA Patients Before Treatment Compared With Normal Subjects

Physical and Biochemical Characteristics

The patients with RA and the normal subjects were similar regarding age, sex, and body weight. The percentage of whole body fat was also similar (32±2%, 33±2%, and 34±1% before and after therapy in patients with RA and in normal subjects; P=NS). At baseline, compared with normal sub-
TABLE 2. Serum Lipid, Lipo-, and Apoprotein Concentrations in Patients With RA and in Normal Subjects

<table>
<thead>
<tr>
<th></th>
<th>RA Patients Before Treatment</th>
<th>RA Patients After Treatment</th>
<th>Normal Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.20±0.23</td>
<td>1.30±0.21</td>
<td>1.24±0.10</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.67±0.20</td>
<td>0.80±0.21</td>
<td>0.71±0.09</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.10±0.46</td>
<td>5.00±0.69</td>
<td>5.80±0.17</td>
</tr>
<tr>
<td>LDL</td>
<td>3.24±0.34*</td>
<td>3.39±0.31</td>
<td>3.85±0.15</td>
</tr>
<tr>
<td>HDL</td>
<td>1.44±0.07</td>
<td>1.49±0.19</td>
<td>1.44±0.06</td>
</tr>
<tr>
<td>HDL2</td>
<td>0.69±0.07</td>
<td>0.66±0.13</td>
<td>0.72±0.05</td>
</tr>
<tr>
<td>HDL3</td>
<td>0.72±0.06</td>
<td>0.75±0.11</td>
<td>0.73±0.02</td>
</tr>
<tr>
<td>Apo A-I, mg/dL</td>
<td>136±7</td>
<td>148±6</td>
<td>147±4</td>
</tr>
<tr>
<td>Apo A-II, mg/dL</td>
<td>34±3</td>
<td>36±2</td>
<td>35±1</td>
</tr>
<tr>
<td>Apo B, mg/dL</td>
<td>94±9</td>
<td>96±11</td>
<td>98±4</td>
</tr>
</tbody>
</table>

Data are shown as mean±SEM. *P<0.05 for patients with RA before treatment vs normal subjects.

projects, the patients with newly diagnosed RA had higher serum C-reactive protein and TNF-α concentrations and erythrocyte sedimentation rates (Table 1). Serum lipid and lipoprotein concentrations are shown in Table 2. Compared with normal subjects, the patients with RA had significantly lower LDL cholesterol concentrations.

**Vascular Function**

Basal blood flows were comparable between the patients with RA and the normal subjects (2.1±0.2 mL/dL per minute for the RA group versus 1.7±0.2 mL/dL per minute in normal subjects, P=NS). The blood flow responses (fold increase in flow in experimental arm above control arm) to low-dose SNP were 30% lower in the RA patients versus normal patients (0.6-fold versus 0.4-fold, respectively), and the responses to high-dose SNP were 34% lower in the RA patients versus normal patients (5.1±0.6-fold versus 7.7±0.7-fold, respectively; P<0.001 by ANOVA). The responses to low-dose ACh were 50% lower in the RA patients versus normal subjects (3.0±0.5-fold versus 6.6±0.7-fold, respectively), and the responses to high-dose ACh were 37% lower in the RA patients versus normal subjects (5.0±0.4-fold versus 7.9±0.8-fold, respectively; P<0.001 by ANOVA; Figure). Within the group of patients with RA, the flow response to ACh was inversely correlated with the erythrocyte sedimentation rate (r=-0.56, P<0.05).

**Effect of Therapy**

**Physical and Biochemical Characteristics**

Body composition did not change during therapy (Table 1). After 6 months of therapy, clinical and laboratory markers of inflammation had significantly decreased (Table 1). After therapy, the concentration of serum LDL cholesterol had increased slightly and was no longer significantly lower in the patients with RA than in the normal subjects (Table 2). The concentrations of other lipids, lipoproteins, and apoproteins were comparable between the groups.

Forearm blood flow responses to intra-arterial SNP (top) and ACh (bottom) infusions in patients with RA before (closed circles) and after (closed triangles) anti-inflammatory therapy and in normal subjects (open circles). ××××P<0.001 for patients with RA before therapy vs normal subjects (ANOVA for repeated measures). *P<0.05 and **P<0.01 for comparison of patients with RA before therapy vs normal subjects at individual doses of ACh and SNP.

**Vascular Function**

Basal blood flow did not change during therapy (2.1±0.1 versus 2.0±0.1 mL/dL per minute for RA before versus after therapy, respectively; P=NS). After therapy, endothelial function improved significantly, as judged from blood flow responses to low and high doses of ACh (P=0.02 for repeated measures by ANOVA, Figure). The blood flow responses to SNP increased slightly but not significantly (Figure). After therapy, the responses to ACh (P=0.33) or SNP (P=0.062) were no longer significantly lower than those in the normal subjects (Figure).

**Discussion**

In the present study, we tested the hypothesis that RA is characterized by in vivo vascular dysfunction by measuring blood flow responses to intra-arterial infusions of ACh and SNP. The measurements were performed in DMARD naive patients with newly diagnosed RA and were repeated 6 months later when the patients were on anti-inflammatory and/or DMARD therapy. The novel finding of the present study was that compared with normal subjects, early untreated patients with RA had blunted vasodilatory responses to the endothelium-dependent vasodilator ACh and the endothelium-independent vasodilator SNP. After 6 months of anti-inflammatory therapy, the vasodilatory responses to ACh had improved significantly. Although increases in the blood flow responses to SNP were not statistically significant, the responses increased and were no longer significantly lower in
the RA patients than in the normal subjects. An impaired vasodilatory response to ACh or shear stress is considered an early abnormality in vascular function preceding atherosclerosis. In the coronary arteries, blunted ACh responses predict cardiac events in patients without obstructive coronary artery disease. In the forearm vascular bed, vasodilatory responses to ACh are blunted in patients with cardiovascular risk factors and are correlated with the severity of coronary artery disease. Autopsy studies, atherosclerosis in the brachial artery has been correlated with atherosclerosis in carotid and coronary arteries.

The blunted vasodilatory responses in the patients with RA before therapy imply a decrease in NO bioavailability. This decrease may be caused by decreased expression of endothelial cell NO synthase (eNOS), lack of substrate or cofactors for eNOS, alteration in the signaling pathways activating eNOS, or accelerated degradation of NO by reactive oxygen species. Given that the responses to ACh and SNP were reduced, the latter mechanism or alternatively blunted responsiveness of smooth muscle to endogenous and exogenous NO could be responsible. The best documented radical that can react with NO is superoxide. The production of superoxide is stimulated by cytokines in several cell types, including vascular smooth muscle cells, and has been documented in human RA. However, acutely, cytokines appear to decrease only endothelin-dependent vasodilatation. In normal subjects, generation of an acute systemic inflammatory response by Salmonella typhimurium attenuates vasodilatory responses to bradykinin and ACh but not to the endothelium-independent agonists verapamil and nitroglycerin. In human veins, acute local application of TNF-α and interleukin-1β impairs vasodilatory responses to bradykinin and arachidonic acid but does not affect the vasodilator response of nitroglycerin. The present data demonstrating impairment in ACh and SNP responses suggest that the effects of chronic inflammation in humans differ from the effects of acute interventions with cytokines on vascular function or that mechanisms other than accelerated NO degradation, such as defects in the response of vascular smooth muscle to NO, contributed to vascular dysfunction.

In the present study, the patients received various antiarthritic and anti-inflammatory therapies, including methotrexate, low doses of corticosteroids, and nonsteroidal anti-inflammatory agents. When patients were studied 6 months later on these treatments, restoration of the blood flow response was observed (especially response to ACh). Because of the small number of patients studied, it is not possible to determine which therapy was most responsible, but this finding is reminiscent of a recent report of restoration of vascular endothelial function in primary systemic vasculitis by immunosuppressive therapy consisting of cyclophosphamide and methylprednisolone. The blood flow response to SNP increased slightly and, after treatment, was no longer significantly lower than that in the normal subjects. The significant increase in the ACh response but not the SNP response suggests that treatment increased NO bioavailability but not the sensitivity of vascular smooth muscle to NO.

The patients with RA had lower LDL cholesterol levels than did the normal subjects, which is in line with previous data. Despite lower LDL cholesterol concentrations, the patients with RA had blunted blood flow responses to ACh compared with responses in normal subjects before therapy. The concentration of LDL cholesterol is one of the major determinants of endothelial function, and its lowering by drugs such as statins improves endothelium-dependent vasodilatation. In the present study, serum LDL cholesterol, if anything, was slightly increased by therapy (Table 2), indicating that other factors are likely to explain the improvement in endothelial function. On the other hand, we did not determine whether LDL was normal regarding its size and other characteristics, such as oxidizability. Recently Hurt-Camejo et al reported that RA patients have higher levels of small dense LDL despite a lower concentration of total LDL cholesterol, LDL particles from RA patients also had significantly higher binding affinity to glycosaminoglycans, suggesting that LDL particles may become trapped in the vessel wall matrix and be prone to oxidation. Modified but not native LDL inhibits endothelium-dependent vascular relaxation.

Data have been lacking regarding vascular function in chronic inflammatory conditions other than those primarily affecting the vascular wall, such as thromboangiitis obliterans, Kawasaki disease, Wegener’s granulomatosis or polyarteritis nodosa, and primary Raynaud’s phenomenon. The present data add RA to the list of diseases characterized by vascular dysfunction. Early suppression of systemic inflammation in RA not only diminishes disease activity but also appears to improve vascular function and may therefore decrease the risk of cardiovascular complications of these patients.

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References


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