Paraoxonase Polymorphism (Gln192Arg) as a Determinant of the Response of Human Coronary Arteries to Serotonin

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Background—Oxidation of LDL plays a role in endothelial dysfunction. Paraoxonase, an enzyme present on HDL, protects LDL against oxidation. Paraoxonase activity is genetically determined in part, and 3 genotypes have been described with variable enzymatic activity. We hypothesized that the paraoxonase polymorphism might influence endothelial function.

Methods and Results—Twenty-seven patients with clinical manifestations of coronary artery disease underwent provocative testing by intracoronary administration of serotonin. None of the coronary arteries studied had significant (>50%) stenosis. Ten patients had the QQ genotype and 17 had the QR genotype. At proximal segments, the mean percentage reduction in lumen diameter in response to serotonin was greater in QQ patients than in QR patients (10^{-5} mol/L; \( P < 0.05 \); 10^{-4} mol/L; \( P < 0.006 \)). Similarly, at distal segments, constriction in response to serotonin was greater in QQ patients than in QR patients (10^{-6} mol/L; \( P < 0.03 \); 10^{-7} mol/L; \( P < 0.07 \)).

Conclusions—These results suggest a higher synthesis or release of endothelium-derived relaxing factors to counteract the vasoconstrictor effect of serotonin in patients with the R allele. These findings provide evidence that the paraoxonase polymorphism may play a role in the regulation of coronary vasomotor tone. (Circulation. 2000;101:740-743.)

Key Words: arteries ■ coronary disease ■ vasoconstriction ■ endothelium

Endothelial dysfunction is an important player in the pathogenesis of coronary artery disease.1 Although the determinants of endothelial dysfunction are largely unknown, in vitro studies2,3 and the improvement in endothelium-dependent vasodilation associated with cholesterol-lowering and antioxidant therapy4 suggest that LDL cholesterol and, in particular, its oxidative derivatives are injurious to the endothelium. It has been suggested that paraoxonase, an enzyme present on HDL, may play a role in oxidative modifications of LDL.5 Paraoxonase activity is in part genetically determined.6—8 A polymorphism (Gln192Arg) based on 2 alleles results in 3 genotypes with variable enzymatic activity.

We hypothesized that the paraoxonase polymorphism might influence endothelial function. In the present study, we analyzed the impact of the paraoxonase Gln192Arg polymorphism on the response of human coronary arteries to serotonin, an endothelium-dependent agonist.

Methods

Patients

Between March and November 1991, 32 patients who underwent coronary angiography in our institution had provocative testing by intracoronary administration of serotonin. These patients were included in 2 prospective studies analyzing the effects of serotonin on coronary vasomotion in patients with coronary artery disease (CAD).9,10 Regular antianginal medication was discontinued 48 hours before catheterization. All patients were taking aspirin (100 to 300 mg daily), which was continued. Patients were allowed to use sublingual nitroglycerin as needed, but no study was performed within 3 hours of its administration.

In January 1996, all patients were contacted by telephone and asked to participate in a study looking for relationships between genetic factors and coronary vasomotion. Two patients had died during the follow-up period, 2 refused to participate, and 1 was lost to follow-up; the 27 remaining patients agreed to undergo venous blood sampling for genetic analysis (see below). These 27 patients form the study population. No significant differences in coronary vasomotion in response to serotonin were observed between these 27 patients and the 5 nonparticipants.

Angiography and Provocative Testing

After diagnostic angiography, an optimal view was chosen to visualize the coronary artery to be studied, and the position of the camera subsequently remained unchanged. In all cases, the coronary artery studied had no significant (>50%) stenosis.

All infusions were administered through 8F-catheters at a rate of 1 mL/min. The patients received a 2-minute infusion of vehicle solution (0.9% saline) followed by 2-minute infusions of serotonin creatine sulfate (10^{-6} through 10^{-5} mol/L). An intracoronary bolus dose of isosorbide dinitrate (ISDN) (2 mg in 2 mL of saline) was injected at the end of the protocol. Coronary angiography was performed at baseline and after each infusion.
Quantitative Coronary Angiography

The coronary angiograms were analyzed with the CAESAR system.\(^6\) The angiographic catheter was used for calibration. The mean diameters of proximal and distal segments, identified by their distance from side branches or from the origin of the vessel, were determined. All measurements were made by a single investigator who was unaware of the design of the study protocol.

Genetic and Biochemical Analyses

Genomic DNA was extracted from white blood cells. The DNA fragment containing the Gln192Arg mutation was amplified and digested with AlwI as described elsewhere.\(^6\) A serum sample drawn at follow-up was used to measure basal paraoxonase activity and arylesterase activity according to previously published protocols.\(^11\)

Statistical Analyses

Statistical analyses were performed with the SAS software release 6.11 (SAS Institute Inc). Mean values ± SD were calculated for quantitative data. The nonparametric Wilcoxon test was used to compare quantitative data, \(t\)- and Fisher’s exact tests were used to compare qualitative data, and nonparametric Spearman correlation coefficients were computed. The effect of serotonin on coronary arteries was expressed as a percentage of variation (positive for vasodilation, negative for vasoconstriction) of luminal diameter from baseline. Standard errors of the mean (SEM) were used to plot quantitative data.

Results

The baseline characteristics of the study population are listed in the Table. The frequencies of the paraoxonase Q and R alleles were 0.68 and 0.32, respectively; 10 patients had the QQ genotype, and 17 had the QR genotype. The baseline characteristics did not differ significantly between the 2 genotypes (Table). As expected, paraoxonase activity was higher in QR patients than in QQ patients; arylesterase activity was similar in both groups. Proximal and distal baseline segment diameters were similar in both groups (QQ: proximal=3.41±0.70 mm, distal=2.27±0.35 mm; QR: proximal=3.27±0.47 mm, distal=2.09±0.44 mm).

The changes observed in the vessel segments we studied are shown in Figure 1. The intracoronary infusion of 0.9% saline was not associated with significant changes in epicardial lumen diameter. In the whole cohort, proximal and distal segments constricted in response to serotonin and relaxed after ISDN. At proximal segments, the mean percentage reduction in lumen diameter in response to serotonin was greater in QR patients than in QQ patients (10\(^{-3}\) mol/L: \(P<0.05\); 10\(^{-4}\) mol/L: \(P<0.006\)). Similarly, at distal segments, constriction in response to serotonin was greater in QQ patients than in QR patients (10\(^{-6}\) mol/L: \(P<0.03\); 10\(^{-3}\) mol/L: \(P<0.07\)). Relaxation after injection of ISDN was similar in QQ patients and QR patients.

Figure 2 shows the plots of vasoconstriction of proximal segments to 10\(^{-4}\) mol/L serotonin versus paraoxonase and arylesterase activities. Although there was a significant \((P=0.02)\) relationship between vasomotion and paraoxonase activity for the overall study population, no apparent relationship between vasomotion and either paraoxonase or arylesterase activity was observed when the patients were divided on the basis of their genotype.

Baseline Characteristics of Study Population

<table>
<thead>
<tr>
<th></th>
<th>QQ (n=10)</th>
<th>QR (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59±11</td>
<td>58±10</td>
</tr>
<tr>
<td>Male, n</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>26±4</td>
<td>25±3</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>214±29</td>
<td>219±36</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>124±25</td>
<td>126±24</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>199±155</td>
<td>188±138</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Smokers</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non–Q wave MI (1–3 weeks before serotonin infusion)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Unstable angina (1–3 weeks before serotonin infusion)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Stable angina</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Asymptomatic (positive treadmill test)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Number of vessels diseases</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0 (nonsignificant)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>62±9</td>
<td>67±13</td>
</tr>
</tbody>
</table>

Vessel studied

- Right coronary artery
- Left anterior descending artery
- Left circumflex artery
- Arylesterase activity, U/mL\(*\)
  - QQ: 99±20
  - QR: 90±16
- Paraoxonase activity, U/mL\(*\)
  - QQ: 107±25
  - QR: 351±84\(|P<0.0001|\)

\(*\) Number of subjects is given for qualitative data. MI indicates myocardial infarction.

Discussion

The healthy endothelium, in part by the release of endothelium-derived relaxing factors (EDRFs), has an important role in maintaining vascular integrity.\(^1\) Several studies have demonstrated that serotonin, a major product of platelet aggregation, can both dilate and constrict the coronary vessels of various species, its net effect being related to the presence or absence of normal endothelium.\(^12\) When infused in normal human coronary arteries, serotonin induces vasodilation, which is thought to be mediated through the release of EDRFs from the endothelium.\(^13\) When infused into the coronary arteries of patients with CAD (as in the present study), serotonin induces a dose-dependent vasoconstriction.\(^14\) It has been postulated that this vasoconstriction reflects endothelial dysfunction, the endothelium of such patients being unable to synthesize or release EDRFs, thus unmasking the direct vasoconstricting effect of serotonin on the underlying vascular smooth muscle.\(^13\)

The results of the present study suggest a higher degree of endothelial dysfunction in patients with the Q allele. We hypothesized that the paraoxonase polymorphism could af-
fect endothelial function because of differences in LDL oxidation, because (1) oxidized LDL particles are more effective than native LDL particles in impairing endothelium-dependent relaxation to acetylcholine, and (2) a randomized, controlled study has demonstrated that coronary artery endothelial dysfunction can be significantly improved by a combination of LDL-lowering and antioxidant therapy. Recent in vitro studies, however, suggest that HDL from individuals with the Q allele confers greater protection against LDL oxidation. This paradoxical observation may suggest that the regulation of LDL oxidation in vivo differs from what is observed in in vitro experiments. Alternatively, we cannot exclude the possibility that the biological mechanism by which the paraoxonase polymorphism influences coronary vasomotion may be unrelated to LDL oxidation.

We found that arylesterase/paraoxonase activities did not correlate with the response to serotonin when the patients were divided on the basis of their genotype. This is not surprising, because it has been shown that the paraoxonase active site required for its arylesterase/paraoxonase activities is different from that required for other activities, such as protection against LDL oxidation. These results concerning enzymatic activities, however, should be taken with caution owing to the retrospective nature of our work and to the limited number of patients for subgroup analysis.

The development of atherosclerosis is a complex process involving multiple potential mechanisms that may differ between populations. Indeed, although recent epidemiological studies have found the R allele to be a risk factor for the development of CAD (reviewed in Reference), the findings of the present study, which included only patients with established CAD, suggest that the Q allele is associated with a greater degree of endothelial dysfunction. In summary, our findings suggest that the paraoxonase polymorphism may play a role in the regulation of coronary vasomotor tone and further demonstrate that vasomotion studies may constitute a new approach for examining the relationship of the human paraoxonase and other polymorphisms to CAD.

References


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