Insulin resistance affects endothelium-dependent acetylcholine-induced coronary artery response

T. Inoue, R. Matsunaga, Y. Sakai, I. Yaguchi, K. Takayanagi and S. Morooka

Department of Cardiology, Koshigaya Hospital, Dokkyo University School of Medicine, Saitama, Japan

Aims This study was designed to investigate the relationship between insulin resistance and the acetylcholine-induced endothelium-dependent coronary artery response in patients without angiographically significant atherosclerotic coronary artery disease and to elucidate the pathophysiological significance of insulin resistance in the early stages of coronary atherosclerosis.

Methods and Results Insulin resistance was calculated from fasting plasma glucose and insulin concentration using homeostasis model assessment in 40 patients suspected of having ischaemic heart disease, but without angiographic evidence of atherosclerotic coronary artery disease defined as a discrete stenosis or intimal irregularity. They were selected for an acetylcholine provocation test in both left and right coronary arteries. The homeostasis model assessment level was higher in 16 acetylcholine-positive patients than in 24 acetylcholine-negative patients (1.84 ± 1.24 vs 0.72 ± 0.62, P < 0.01). Comparisons of the percentage change in vessel lumen diameter after the acetylcholine test in each of proximal, mid and distal segments of three coronary arteries among the three groups of low (less than 0.7; n=13), intermediate (0.7 to 1.4; n=13), and high homeostasis model assessment level (more than 1.4; n=14) revealed that a higher level resulted in a worse acetylcholine-induced constrictive response in coronary arteries.

Conclusion These results suggest that there is an association between high insulin resistance and coronary vascular endothelial cell dysfunction, and that insulin resistance may be an indicator of early stage coronary artery atherosclerosis not detectable by angiography. (Eur Heart J 2000; 21: 895–900)

Key Words: Acetylcholine, coronary artery vasospasm, endothelial dysfunction, homeostasis model assessment, insulin resistance, risk factors.

Introduction

It has been shown that acetylcholine dilates normal arteries by stimulating the release of endothelium-derived relaxing factor[1,2]. Acetylcholine also constricts vascular smooth muscle, and the net acetylcholine response is believed to result from these opposing actions[3]. When the endothelium is removed or dysfunctional, the vasodilator response is impaired and the vasoconstrictor response becomes dominant[3]. Vasoconstriction is considered to be the extreme response of vasoconstriction. Hence, impaired acetylcholine-induced vasodilation may reflect endothelial dysfunction and is likely to be an indicator of early stage atherosclerosis[4].

The impairment of the vasodilator response to acetylcholine has been shown in animal atherosclerosis models and in atherosclerotic human coronary arteries studied in vitro[3,5,6]. Various coronary risk factors are known to impair endothelium-dependent vasodilation in experimental studies[7–12].

Hyperinsulinaemia or insulin resistance is known to be important in the aggravation of established coronary risk factors, and recently has been shown to contribute to the development of coronary artery disease[13–15]. However, whether insulin resistance affects the endothelium-dependent coronary artery response remains unknown. This study was designed to investigate the relationship between insulin resistance and acetylcholine-induced endothelium-dependent coronary artery response in patients without angiographically significant atherosclerotic coronary artery disease, and to elucidate the pathophysiological significance of insulin resistance in the early stages of coronary atherosclerosis.
Methods

Patient selection

We performed sampling of fasting blood in 648 consecutive patients who underwent diagnostic cardiac catheterization with coronary angiography early in the morning of the study day. Among these patients, we selected 40 patients (26 men and 14 women aged 59±9 years) suspected of having ischaemic heart disease, but without angiographically significant atherosclerotic coronary artery disease, defined as a discrete stenosis or intimal irregularity, as well as any other heart disease. These patients underwent acetylcholine provocation tests in both the left and right coronary arteries. Patients who had diabetes mellitus requiring hypoglycaemic agents, or who had been receiving lipid lowering drugs, were excluded. In all patients, all oral medications except sublingual nitroglycerin were discontinued at least 48 h before angiography. The study protocol was approved by the Dokkyo University Institutional Review Board, and written informed consent was obtained from each patient.

Coronary angiography and acetylcholine test

Coronary angiography was performed in the morning of the study day using the Judkins technique with a 6F Judkins catheter (Bard, Billerica, MA, U.S.A.). Blood pressure was monitored through the catheter, and a standard 12-lead electrocardiogram was recorded during the study with a six-channel recorder. After intravenous bolus injection of 5000 IU of heparin, single-plane coronary cineangiograms were obtained at 30 frames/second, following injection of non-ionic contrast material (Iomeron 350, Eisai Co., Ltd., Tokyo, Japan). After the baseline left and right coronary angiograms had been obtained, serial doses of 40 and 80 μg of acetylcholine and of 30 and 50 μg of acetylcholine, respectively, were injected into the left and right coronary arteries over a 60-s period. Angiograms were obtained 90 s after the start of each injection. When ST-segment changes, chest pain, or both appeared after the acetylcholine injection, angiography was performed immediately. When a significant coronary vasospasm occurred during the first dose of acetylcholine (30 μg for the right coronary artery or 40 μg for the left coronary artery), injection of the second dose (50 μg for the right coronary artery or 80 μg for the left coronary artery) was not performed. In this study, a positive acetylcholine test was defined as the occurrence of significant coronary vasospasm (segmental or diffuse luminal narrowing of more than 90% or total occlusion of the artery) with marked ST-T changes and/or chest pain.

Quantitative angiographic analysis

Coronary artery lumen diameters were measured with the use of an automated edge contour detection computer system (CAM-1000 system, PSP Corp, Tokyo, Japan), from end-diastolic film frame. The diameters of proximal, mid and distal segments of the left anterior descending artery, the left circumflex artery and the right coronary artery were measured. The measured segments were based on the side branches, and identical segments were analysed on the same projection before and after the acetylcholine test. The size of the Judkins catheter was used to calibrate the image, and correction was made for radiographic pincushion distortion. coronary arteries inadequately opacified and segments overlapped by other structures were not analysed. The lumen diameter was determined for each proximal, mid, and distal segment, and percentage changes in the diameter (% change), by acetylcholine response, were calculated for each segment as follows: % change=(Da−Db)/Db×100, where Da=diameter after the acetylcholine test, and Db=diameter before the acetylcholine test. If the vessel lumen was occluded in the measured segment by coronary artery spasm, the Da was defined as zero. These methods for analyses have been described and validated in a previous study[11]. The analyses were performed by one investigator who was unaware of the identity of the patient and of the working hypothesis. Intra-observer variability for the measurement of the coronary artery diameter showed high reproducibility (r=0.98, SEE=3.6%, P<0.001).

Measurements

Fasting venous blood was taken from the antecubital vein early in the morning on the day of coronary angiography for determination of plasma glucose and insulin levels, serum total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride, apolipoprotein (apo) E, apo A-I and apo B, and lipoprotein(a). Insulin resistance was determined from fasting plasma glucose (mmol.L⁻¹) and insulin concentrations (mU.L⁻¹), using homeostasis model assessment, by the formula [resistance=insulin/(22.5×e⁻ln glucose)] as described previously; this was correlated with insulin resistance measured using a euglycemic clamp[19]. In this study, the homeostasis model assessment levels were divided into ‘low’ (less than 0.7), ‘intermediate’ (0.7 to 1.4), and ‘high’ (more than 1.4).

Statistical analysis

Values were expressed as mean ± SD. Intra-group comparisons were analysed with the use of paired Student’s t-tests. Inter-group comparisons between two groups were performed with the use of unpaired t-tests for continuous variables and of chi-square tests for categorical variables. Comparisons among the three groups were analysed using one-way analysis of variance (ANOVA). A P value of less than 0.05 was considered to be significant.
Table 1  Comparison of baseline characteristics and coronary risk factors between acetylcholine-positive and negative groups

<table>
<thead>
<tr>
<th></th>
<th>ACh positive (n=16)</th>
<th>ACh negative (n=24)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60 ± 9</td>
<td>58 ± 9</td>
<td>ns</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>10/6</td>
<td>16/8</td>
<td>ns</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4 (25%)</td>
<td>5 (21%)</td>
<td>ns</td>
</tr>
<tr>
<td>Smoking</td>
<td>10 (62%)</td>
<td>12 (50%)</td>
<td>ns</td>
</tr>
<tr>
<td>Glucose (mg. dl⁻¹)</td>
<td>94 ± 26</td>
<td>104 ± 18</td>
<td>ns</td>
</tr>
<tr>
<td>Insulin (mU . l⁻¹)</td>
<td>7.0 ± 6.2</td>
<td>2.9 ± 1.9</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.94 ± 1.24</td>
<td>0.72 ± 0.62</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>TC (mg. dl⁻¹)</td>
<td>201 ± 29</td>
<td>198 ± 27</td>
<td>ns</td>
</tr>
<tr>
<td>TG (mg. dl⁻¹)</td>
<td>142 ± 58</td>
<td>139 ± 64</td>
<td>ns</td>
</tr>
<tr>
<td>HDL-C (mg. dl⁻¹)</td>
<td>48 ± 22</td>
<td>51 ± 21</td>
<td>ns</td>
</tr>
<tr>
<td>Apo A-I (mg. dl⁻¹)</td>
<td>121 ± 18</td>
<td>109 ± 25</td>
<td>ns</td>
</tr>
<tr>
<td>Apo B (mg. dl⁻¹)</td>
<td>97.7 ± 23</td>
<td>87.2 ± 22</td>
<td>ns</td>
</tr>
<tr>
<td>Apo E (mg. dl⁻¹)</td>
<td>3.5 ± 0.9</td>
<td>3.6 ± 0.8</td>
<td>ns</td>
</tr>
<tr>
<td>Lp(a) (mg. dl⁻¹)</td>
<td>22 ± 21</td>
<td>25 ± 35</td>
<td>ns</td>
</tr>
</tbody>
</table>

ACh=acetylcholine; HOMA=homeostasis model assessment; TC=total cholesterol; TG=triglyceride; HDL-C=high density lipoprotein-cholesterol; Lp(a)=lipoprotein(a); Apo=apolipoprotein.

Results

Results of the acetylcholine test

For all 40 patients, the maximal response in the % change in the lumen diameter in each segment of coronary artery after the acetylcholine tests ranged from −86% (constriction) to +26% (dilation) in the proximal segment, from −100% (total occlusion) to +44% in the mid segment, and from −100% to −78% in the distal segment of the left anterior descending artery; from +78% to +58% in the proximal segment, from −100% to +42% in the mid segment, and from −100% to +54% in the distal segment of the left circumflex artery; from −100% to +45% in the proximal segment, from −100% to +52% in the mid segment, and from −100% to +68% in the distal segment of the right coronary artery. Sixteen of the 40 patients showed positive results in the acetylcholine test. Coronary vasospasm in a single vessel was seen in 10 patients, in two vessels in five patients, and in three vessels in one patient. Thus, a total of 21 vessels indicated significant vasospasm by the acetylcholine test. Vasospasm was induced by the first dose of acetylcholine in 11 of the 21 total vessels, and by the second dose in the remaining 10 vessels.

Relationship between insulin resistance and acetylcholine-induced coronary vascular response

The mean levels of fasting plasma glucose and insulin in the 40 patients were 102 ± 21 mg. dl⁻¹ (range 72–151 mg. dl⁻¹) and 4.6 ± 4.6 mU. l⁻¹ (range 0.5–23.1 mU. l⁻¹), respectively. The insulin resistance by homeostasis model assessment was 1.18 ± 1.30 (range 0.06–6.27).

Between the 16 acetylcholine-positive patients and the remaining 24 acetylcholine-negative patients, there were no significant differences in age, gender, hypertension, smoking habits, or any underlying heart conditions. The levels of fasting plasma glucose, total cholesterol, triglyceride, HDL-cholesterol, apo A-I, apo B, apo E, and lipoprotein(a) were also similar between the two groups. However, plasma insulin levels (7.0 ± 6.2 vs 2.9 ± 1.9 mU. l⁻¹, P<0.01) and homeostasis model assessment levels (1.84 ± 1.24 vs 0.72 ± 0.62, P<0.01) were higher in the acetylcholine-positive group than in the acetylcholine-negative group (Table 1).

Thirteen of 40 patients had low homeostasis model assessment levels (mean 0.40 ± 0.18), another 13 had intermediate levels (mean 1.20 ± 0.44), and the remaining 14 had high levels (mean 2.48 ± 1.51). In the low level group, the vessel lumen diameter did not change significantly after the acetylcholine test in the proximal (3.22 ± 0.32 to 3.14 ± 0.54 mm) or mid segments (2.51 ± 0.43 to 2.54 ± 0.58 mm), but slightly increased in the distal segment (1.75 ± 0.72 to 1.94 ± 0.81 mm, P<0.05) of the left anterior descending artery. It also did not change in the proximal (2.96 ± 0.52 to 2.87 ± 0.63 mm) or mid segments (2.06 ± 0.68 to 2.03 ± 0.72 mm), but also slightly increased in the distal segment (1.58 ± 0.74 to 1.72 ± 0.81 mm, P<0.05) of the left circumflex artery. The segments of the right coronary artery did not change (3.18 ± 0.78 to 3.23 ± 0.84 mm) in the proximal, 2.67 ± 0.86 to 2.73 ± 0.94 mm in the mid, and 2.02 ± 0.94 to 2.07 ± 1.06 mm in the distal segments.

In the intermediate homeostasis model assessment group, the diameter of the anterior descending artery slightly decreased after the acetylcholine test in the proximal segment (3.18 ± 0.38 to 2.79 ± 0.62 mm, P<0.05), but did not change significantly in the mid (2.49 ± 0.46 to 2.38 ± 0.58 mm) or distal segments.
arteries become stronger in relation to higher homeostasis model assessment levels. This reveals a dose-related effect in that the constrictive response of the coronary arteries became stronger in relation to higher homeostasis model assessment levels. *P<0.05, **P<0.01.

Figure 1 shows the % change in the lumen diameter after the acetycholine test in each of the proximal, mid, and distal segments in three coronary arteries among three groups of low (□), intermediate (□□), and high (□□□) homeostasis model assessment groups. This revealed a dose-related effect in that the constrictive response of the coronary arteries became stronger in association with higher homeostasis model assessment levels.

In 16 acetycholine-positive patients, there was total vessel occlusion of some segments of the coronary arteries after the acetycholine test in six patients. Among them, four patients were in the high level group, and the remaining two in the intermediate group. No patients in the low level group had total occlusion.

Discussion

In this study, we avoided a heterogeneous patient population and carefully selected patients without angiographic evidence of atherosclerotic coronary artery disease. All patients underwent acetycholine provocation testing in the same manner. In the patients of our study, coronary risk factors including hypertension, hyperlipidaemia, and smoking were similar in the acetycholine-positive and negative groups. Fasting plasma glucose levels were also similar in both groups. However, the fasting plasma insulin level and insulin resistance determined by homeostasis model assessment were higher in the acetycholine-positive group than in the acetycholine-negative group. In addition, we found that endothelium-dependent acetycholine-induced coronary artery response had become more constrictive in association with higher homeostasis model assessment levels.
Since 1980, it has been known that acetylcholine-induced vasodilation was dependent on the vascular endothelium\[17\]. This led to the suggestion that acetylcholine stimulates the release of a substance from endothelial cells, endothelium-derived relaxing factor, which is nowadays identified as nitric oxide, and diffuses to the underlying smooth muscle cells to cause relaxation. Thus, impaired vascular relaxation in response to acetylcholine is thought to reflect endothelial dysfunction\[19\]. Coronary vasoconstruction induced by acetylcholine is attributed to its direct action on vascular smooth muscle cells and can arise when the endothelium is damaged or its function impaired\[1,4\]. Intracoronary ultrasound findings indicate that atheromatous plaque is present even in lesions where the endothelium-dependent vasodilation response is impaired in spite of the absence of angiographic evidence of atherosclerosis\[19\]. Therefore, impaired endothelium-dependent vasodilatation response is believed to be an early manifestation of atherosclerosis.

Among well-known coronary risk factors, abnormalities of glucose metabolism as well as lipid metabolism are closely related to the development of coronary atherosclerosis. Recently, hyperinsulinaemia or insulin resistance has been emphasized as a risk factor for atherosclerotic coronary artery disease\[20,21\]. In addition, insulin infusion in healthy volunteers is associated with vasodilation possibly induced by endothelium-derived nitric oxide. However, insulin-induced nitric oxide production is lacking in insulin-resistant subjects. Hyperinsulinaemia induces hypertension, which may be caused by increased renal sodium retention\[22\], increased Na\(^+\)-H\(^+\) exchange in vascular wall cells\[23\], or increased sympathetic activity\[24\]. Hyperinsulinaemia also induces abnormalities of lipid metabolism\[25\]. In addition to this atherogenic evidence, insulin itself may proliferate vascular smooth muscle cells mediated by stimulating insulin-like growth factor receptors\[26\], leading to atherosclerosis. The homeostasis model assessment is a simple method of assessing insulin resistance and beta-cell function in fasting plasma glucose and insulin concentrations. Recently, this method has been evaluated extensively, and the estimate of insulin resistance obtained by homeostasis model assessment seems to be correlated with that obtained by the euglycemic clamp method. However, it is suggested that insulin resistance by homeostasis model assessment is unlikely to be precise in patients with marked hyperglycaemia. Therefore, our study excluded such patients.

Our results suggest that coronary endothelial damage or dysfunction, namely early stage coronary atherosclerosis, may be present in association with hyperinsulinaemia or high insulin resistance in patients without angiographically significant coronary artery lesions.

References

