Original Article

Impact of Paraoxonase Polymorphism (Q192R) on Endothelial Function in Intact Coronary Circulation

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Paraoxonase-1 (PON1) can protect endothelial function by preventing the oxidation of low-density lipoprotein (LDL) cholesterol and retarding the development of atherosclerosis. We examined whether PON1 polymorphism influences endothelium-dependent coronary vasomotor responses. Sixty-seven patients underwent diagnostic cardiac catheterization, but showed no significant coronary artery stenosis. In all patients, PON1 genotypes (Q/Q, Q/R and R/R) were determined, and provocative testing was performed by the intracoronary administration of graded doses of bradykinin (BK; 0.2, 0.6 and 2.0 μg/min) and acetylcholine (ACh; 3, 10 and 30 μg/min). Coronary blood flow (CBF) was evaluated by a Doppler guide wire. The patients were divided into 2 groups on the basis of ACh testing: one with coronary spastic angina (CSA) and one with non-CSA. The frequencies of the PON1 genotype in the CSA group did not differ significantly from those in the non-CSA group. In the non-CSA group, the patients were subdivided into 2 groups: a group with the Q/Q or Q/R genotypes and a group with the R/R genotype. The vasoconstrictive responses of the epicardial coronary artery to ACh were comparable between the Q/Q + Q/R and R/R groups. Also, the coronary vasodilations induced by BK in the R/R group were similar to those in the QR + QQ group. There were no significant differences in the CBF responses induced by BK or ACh between the Q/Q + Q/R and R/R groups. In conclusion, as estimated by BK and ACh testing, our findings suggest that PON1 genotypes may not play a critical role in the modulation of endothelial vasomotor function in the intact coronary circulation. (*Hypertens Res* 2006; 29: 417–422)

Key Words: paraoxonase-1 (PON1), coronary circulation, endothelium, bradykinin

Introduction

The oxidation of low-density lipoprotein (LDL) cholesterol plays a role in the impairment of endothelial function and arterial reactivity (*1*). High-density lipoprotein (HDL) cholesterol has been shown to protect against LDL cholesterol oxidation (*2*). There is considerable evidence that the antioxidant activity of HDL cholesterol is markedly due to paraoxonase-1 (PON1) located on it. There is considerable evidence that

PON1 plays a major role in the antioxidant activity of HDL cholesterol. The PON1 gene has two common coding-region polymorphisms (glutamine → arginine at position 192 and methionine → leucine at position 55), which regulate the concentration and activity of the enzyme and alter its ability to prevent lipid oxidation. Previous studies have shown that polymorphisms of the PON1 are correlated with coronary artery disease (*3–5*).

It is widely accepted that coronary endothelial function is abnormal in patients with coronary atherosclerosis or risk fac-

tors for atherosclerosis. Since an abnormal response to vaso-active stimuli is likely to precede the angiographic recognition of coronary atherosclerotic lesions, it is important to detect changes in endothelial vasoactive function early in the development of atherosclerosis (*6*).

It was recently shown that the PON1-192R allele is significantly associated with coronary vasospasm in the Japanese population (*7*). Several studies have suggested that the PON1 Q192R polymorphism is associated with coronary vasomotor tone (*8*, *9*).

We hypothesized that this PON1 polymorphism might influence coronary endothelial function and vasomotor tone. To test this hypothesis, we examined whether PON1 polymorphism affects endothelium-dependent coronary vasomotor function in patients with no significant stenosis of the coronary arteries. Coronary vascular responses to acetylcholine (ACh) and bradykinin (BK) were examined as a marker of endothelial function (*10–12*).

Methods

Study Patients

The study protocols were approved by the Ethical Committee on Human Research at our institution, and written informed consent was obtained from all patients. The study population included 67 consecutive patients (48 men and 19 women; mean age: 62.7 years; range: 41–80 years) who underwent diagnostic cardiac catheterization, including coronary angiographic study, for the evaluation of chest pain and/or myocardial ischemia on an ECG. Patients with myocardial infarction, congestive heart failure, cardiomyopathy, valvular heart disease, or coronary narrowing exceeding 25% of the luminal diameter of the left anterior descending coronary artery (LAD) were excluded from this study.

Smokers were defined as subjects who were currently using tobacco and had been doing so for more than 1 year. Non-smokers were defined as individuals who had never smoked. Smokers refrained from smoking for at least 7 days before the present study to rule out the direct effects of oxidants contained in cigarette smoke.

All cardiac medications were discontinued for at least 72 h before the study, and aspirin was discontinued for at least 7 days before the study. We divided the subjects into two groups based on the response to ACh: a coronary spastic angina (CSA) and a non-CSA group. The CSA group consisted of 24 patients with angiographically determined coronary artery spasm (total or subtotal occlusion) with completely normal coronary arteries. The non-CSA group was comprised of 43 patients with a negative response to ACh (30 or 100 µg/min).

Graded doses of ACh were infused into the left coronary artery (LCA) (30 or 100 µg/min) and thereafter into the right coronary artery (RCA) (50 µg/min) until coronary spasm was evoked, or the maximal doses were reached in all patients.

Ten minutes after the infusion of ACh into the LCA was complete, when the arterial pressure and the coronary arterial diameter had returned to the baseline level, ACh (50 µg/min) was infused into the RCA.

Protocol

After the diagnostic coronary angiographic study was performed, a 6 Fr Judkins-type guide catheter (Medtronic Inc., Santa Rosa, USA) was introduced into the left main coronary artery, and a 0.014 inch Doppler-tipped guide wire (Jometrics FloWire, JoMed Inc., Rancho Cordova, USA) was advanced through the guide catheter (*13*, *14*). The wire was positioned in the middle segment of the LAD such that there were no major branches within 1.0 cm of the tip. All drugs were infused directly into the left main coronary artery *via* a 2.6 Fr coronary-infusion catheter (Cordis Endovascular System Inc., Miami, USA) at infusion rates ranging between 0.5 and 1.0 ml/min.

Baseline coronary angiography and measurement of coronary blood flow (CBF) velocity were performed. BK- and ACh-induced vasodilation responses were established by dose-response curves obtained with incremental 2-min intracoronary infusions of BK and ACh. Coronary angiography and measurement of CBF velocity were performed after each infusion. BK was started at 0.2 µg/min, and increased to 0.6 and 2.0 µg/min. After completing the protocol with the intracoronary injection of BK, we waited for at least 10 min before beginning the infusion of ACh, by which time the coronary artery diameter and CBF velocity had returned to the baseline values. Baseline coronary angiography and measurement of CBF velocity were performed again. Next, ACh was started at 3.0 µg/min, and increased to 10 and 30 µg/min. When there was no response to ACh at less than 30 µg/min, ACh was administered at 100 µg/min over 90 s, and the presence/absence of coronary artery spasm was determined. Nitroglycerin (NTG) was subsequently injected into the LAD at 250 µg for 20 s. After an additional 10 min, by which time the CBF velocity and aortic pressure had returned to the baseline values, intracoronary administration of papaverine (PA) was performed at 12 mg for 20 s. The maximal CBF velocity was measured after the infusion of PA. Coronary angiography was performed at 2 min after the infusion of NTG or PA. During the study, phasic and mean aortic blood pressure, heart rate, and 12-lead ECG were monitored continuously.

Quantitative Coronary Angiography and Measurements of Coronary Blood Flow

The change in diameter of the LAD was measured in a vessel segment 2.5 mm beyond the tip of the Doppler-wire. Coronary angiograms were analyzed by quantitative coronary angiography using a Cardiovascular Measurement System (QCA-CMS; MEDIS Medical Imaging Systems, Leiden, the Netherlands). Measurements were made 3 times, and the

Table 1. Clinical Characteristics

	Non-CSA group (*n*=43)		*p* value
	Q/Q + Q/R (*n*=20)	R/R (*n*=23)	
Age (years)	63.4±1.7	63.2±2.0	n.s.
Gender (male)	15 (75.0%)	15 (65.2%)	n.s.
Body mass index (kg/m^2)	25.7±0.8	25.3±0.5	n.s.
Hypertension	12 (60.0%)	12 (52.2%)	n.s.
Hyperlipidemia	11 (55.0%)	11 (47.8%)	n.s.
Diabetes	6 (30.0%)	5 (21.7%)	n.s.
Smoking	4 (20.0%)	4 (17.4%)	n.s.
Total cholesterol (mg/dl)	193.4±6.9	192.5±6.9	n.s.
Triglyceride (mg/dl)	140.1±13.1	122.6±9.5	n.s.
HDL cholesterol (mg/dl)	45.3±5.5	41.1±3.1	n.s.
LDL cholesterol (mg/dl)	123.3±7.3	121.8±6.9	n.s.
Coronary diameter(control) (mm)	2.21±0.12	2.34±0.10	n.s.
Mean blood pressure (mmHg)	96.1±4.3	91.5±1.8	n.s.
Heart rate (bpm)	66.5±2.5	66.6±2.3	n.s.

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

average value was used for analysis. Peak CBF velocity was continuously monitored using a fast Fourier transform–based spectral analyzer (FloMap; Cardiometrics Inc., Mountain View, USA). CBF was calculated as

$$CBF = \pi \times \text{average peak CBF velocity} \times 0.125 \times (\text{arterial diameter})^2.$$

Vessel diameter and CBF velocity were analyzed by investigators who were blinded to the sequence of the investigations.

Preparation of Bradykinin

BK (Sigma Chemical Co., St. Louis, USA) was diluted with physiological saline at a concentration of 2 µg/ml and sterilized at the Pharmacy Department of the Shiga University of Medical Science Hospital (Otsu, Japan).

Paraoxonase Genotype Determination

PON1 genotypes were determined using the polymerase chain reaction (PCR) according to previously published protocols with modifications (*15–17*). Genomic DNA was extracted from white blood cells.

The DNA region containing the polymorphic site (a 99-bp DNA fragment) was amplified by PCR using two primers, the sense primer 5′-TATTGTTGCTGTGGGACCTGAG-3′ and antisense primer 5′-CACGCTAAACCCAAATACATCTC-3′, followed by restriction endonuclease (*AlwI*) digestion. The samples were then separated by electrophoresis on 4.5% Nusieve gel and visualized by using ethidium bromide.

Statistical Analysis

Data are expressed as the means±SEM. Discrete variables are expressed as counts or percentages and were compared with the χ^2 test or Fisher's exact test, as appropriate. Continuous variables were compared using the unpaired Student's *t*-test or a one-way analysis of variance (ANOVA). When serious changes in coronary and systemic hemodynamic variables in response to graded doses of drugs were compared, a two-way ANOVA for repeated measures followed by Bonferroni's multiple-comparison test was used within each group. A value of *p*<0.05 was considered statistically significant.

Results

Patient Characteristics and Frequencies of the PON1 Genotypes

All 24 patients in the CSA group showed coronary vasospasm in the LAD coronary artery into which ACh was infused, along with ischemic ECG changes and/or chest pain. Coronary vasospasm was not documented in RCAs. The Q/Q, Q/R, and R/R genotypes were present in 1 (4.2%), 14 (58.3%), and 9 (37.5) of the patients in the CSA group, respectively. Among the 43 patients of the non-CSA group, the Q/Q, Q/R, and R/R genotypes were found in 3 (7.0%), 17 (39.5%), and 23 (53.5%) patients, respectively.

Since there were too few patients to perform analyses using three groups, the data for the QQ and QR genotypes were combined for analyses. We divided the subjects in the non-CSA group into two groups according to whether they had the PON1 genotype with or without the Q allele (Q/Q + Q/R genotype: *n*=20; R/R genotype: *n*=23). The baseline characteristics are shown in Table 1. Age, gender, body mass index, hypertension, hypercholesterolemia, diabetes, smoking and lipid parameters did not differ significantly between the two groups. The diameter of the LAD coronary artery, its average
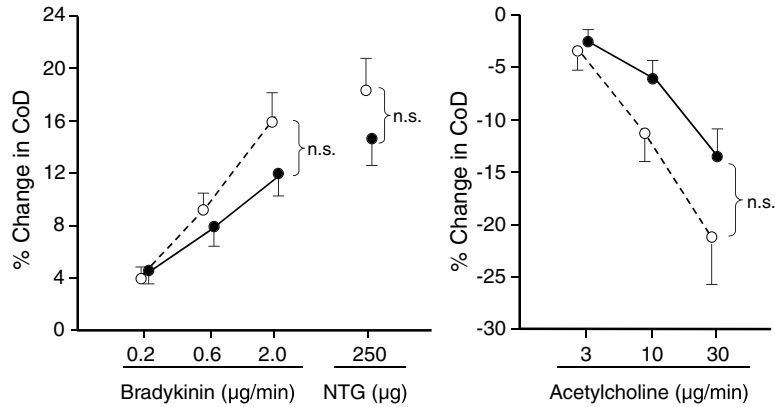
Fig. 1. *Relationship between the paraoxonase-1 polymorphism genotype (Q/Q + Q/R, open circles, n = 20; R/R, closed circles, n = 23) and the percentage change in coronary artery diameter (CoD) induced by bradykinin (left), acetylcholine (right) and nitroglycerin (NTG; left) in patients with no coronary spastic angina (non-CSA group).*
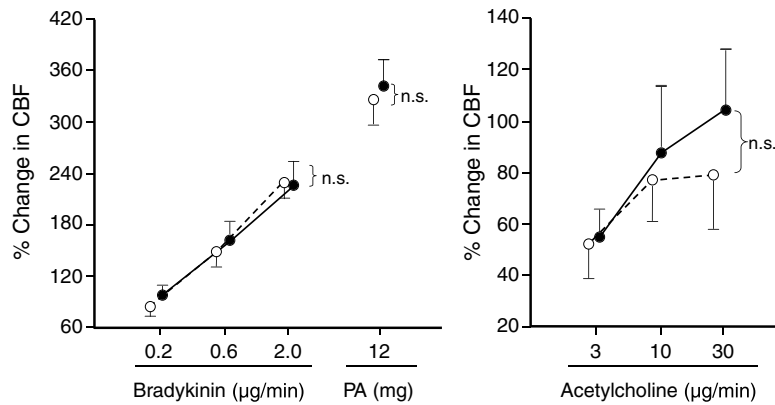

Fig. 2. *Relationship between the paraoxonase-1 polymorphism genotype (Q/Q + Q/R, open circles, n = 20; R/R, closed circles, n = 23) and the percentage change in coronary blood flow (CBF) induced by bradykinin (left), acetylcholine (right) and papaverine (PA; left) in patients with no coronary spastic angina (non-CSA group).*

peak CBF, mean blood pressure and heart rate at baseline also did not differ between these two groups.

Coronary Endothelial Function

The coronary artery diameter (CoD) and the CBF of the LAD coronary artery increased depending on the dose of BK administered. Percentage increases in the diameter of the LAD coronary artery in response to BK infusion at doses of 0.2–2.0 μg/min did not differ significantly between the two groups (Fig. 1). The diameter of the LAD coronary artery decreased depending on the dose of ACh administered. The percentage decrease in diameter in response to ACh infusion at 3.0–30 μg/min did not differ significantly between the two groups (Fig. 1). The percentage changes in NTG-induced vasodilation were comparable between the two groups (Q/Q + Q/R genotype: 19.3±2.4% *vs.* R/R genotype: 13.6±1.6%,

n.s.) (Fig. 1).

The percentage increases in CBF induced by the three doses of BK did not differ between the two groups (Fig. 2). The percentage changes in CBF induced by the three doses of ACh did not differ significantly between the two groups (Fig. 2). The percentage changes in CBF induced by PA were comparable between the two groups (Q/Q + Q/R group: 326.4± 30.9%; R/R group: 343.3±28.2%; n.s.) (Fig. 2).

Discussion

Major Findings

The present study is the first to demonstrate that PON1 polymorphism is not correlated with coronary macro- or microvasomotor responses induced by BK in patients with normal coronary arteries.

PON1 Polymorphism and Coronary Endothelial Function

In the present study, we examined the influence of PON1 polymorphism on coronary effects induced by ACh and BK in patients with normal coronary arteries. ACh has vasodilative action mediated through the release of NO from the endothelium, and also has direct vasoconstricting effects on coronary smooth muscle cells. It has been shown that the intracoronary injection of ACh elicits severe vasoconstriction in patients with coronary vasospasm (*12*). Unlike ACh, BK induces only endothelium-dependent relaxation *via* the action of NO, prostacyclin and hyperpolarizing factor, but does not have a direct vasoconstricting effect on human coronary smooth muscle cells (*10*, *11*). Both agents were used as a pharmacological tool for estimating coronary endothelial function. In the present study, the increases in epicardial coronary artery diameter and CBF in response to BK and ACh were comparable between the Q/Q + Q/R group and the R/R group. We previously reported that coronary macro- and microvasomotor responses induced by BK correlate significantly with the plasma oxidized LDL level (*18*). Increased oxidative stress impairs endothelial function (*19*, *20*). Some reports have suggested that the R allele is a risk factor for the development of coronary artery disease (*3*, *4*, *21*), whereas others have reported that this allele does not pose such a risk (*22*, *23*). In contrast to previous reports and the present study, Bauters *et al*. reported that the Q allele is associated with a greater degree of serotonin-induced epicardial coronary vasoconstriction in patients with coronary artery disease, suggesting that the Q allele, rather than the R allele, may be a risk factor for coronary heart disease (*8*). They showed that the biological mechanism by which PON1 polymorphisms influence coronary vasomotion may be unrelated to LDL oxidation (*8*). Further studies are needed to examine the relationship between PON1 polymorphisms and coronary vasomotion and its mechanism.

It has previously been shown that the arterial vasodilation induced by NTG was inversely related to endothelium-dependent vasodilation (*24*). Oxidative stress can scavenge both endogenous and exogenous NO, thereby decreasing the bioavailability of NO to smooth muscle cells. Thus, the blunted vasodilation in response to NTG may reflect reduced oxidative stress. Furthermore, maximal CBF responses (which reflect a microvascular response) to PA also did not differ between the two groups. Malin *et al*. showed that PON1 polymorphism influences coronary vasomotor tone and, consequently, CBF in young healthy men (*9*). However, in the present study, PON1 polymorphism was not associated with coronary macro- or microsmooth muscle function.

PON1 Polymorphism and Coronary Artery Spasm

Ito *et al*. reported that PON1 polymorphism is related to coronary vasospasm, and suggested that the mechanism for this

relation may be that the PON1-192R allele confers lower antioxidant activity than the PON1-192Q allele, since the plasma thiobarbituric acid reactive substances level, as a marker of oxidative stress, is higher in the R/R genotype of the PON1 gene than in the Q/Q genotype (*7*). Mashiba *et al*. showed a significant association between PON1 A632G polymorphism and both microvascular angina and vasospastic angina as diagnosed by acetylcholine provocation test (*25*). PON1 A632G polymorphism results in amino acid substitution (Q192R), and PON1 with the 632G allele shows reduced antioxidant activity. However, our data lacked sufficient power to address this interaction because of the small sample size. Activities of coronary vasospasm are not merely present or absent, but present to a greater or lesser extent in each individual. In addition, considering the high prevalence of treatment with lipid-lowering and antihypertensive agents, our patients likely had a combination of risk factors. Therefore, it is difficult to assess the effects of PON1 genotypes on coronary vasospasm in the present study population. It has been reported that there is a wide variation in serum PON1 activity within and between populations (*26–28*). Thus, further studies are needed to confirm the significance of PON1 in the genesis of coronary vasospasm.

Conclusion

The present results suggest that PON1 Q192R polymorphism is not associated with coronary endothelial function in the intact coronary circulation.

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