Coexistence of Impairment of Endothelium-Derived Nitric Oxide and Platelet-Derived Nitric Oxide in Patients With Coronary Risk Factors

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Impairment of endothelium-derived nitric oxide (EDNO) has been demonstrated in patients with coronary risk factors in some studies, as well as impaired platelet-derived nitric oxide (PDNO) in other studies. However, no study has examined whether these impairments coexist. In 24 patients with coronary risk factors, femoral vascular endothelial function was assessed with acetylcholine (ACh: 50, 100, 200 and 400 μg/min) and endothelium-independent vascular function with nitroglycerin (NTG; 50, 100, 200 μg/min) using a Doppler flow-wire technique, as well as ADP (5 μmol/L)-induced PDNO release with an NO-specific electrode. The ACh-mediated percent change in femoral vascular resistance index (% change of FVRI) and PDNO release had a significant correlation with the number of risk factors. The ACh-mediated % change of FVRI, but not that with NTG, significantly correlated with the PDNO release. Both EDNO and PDNO bioactivities are impaired in patients with coronary risk factors and there is a common mechanism. (Circ J 2002; 66: 837–840)

Key Words: Bioactivity; Endothelium; Nitric oxide; Platelets; Risk factors

Endothelium-derived nitric oxide (EDNO), which accounts for the major biological properties of the endothelium-derived relaxing factor, is synthesized from L-arginine via NO synthase (NOS). NO is a single molecule with profound effects on cardiovascular physiology: it inhibits pathologic processes such as abnormal arterial vasomotion, thrombosis, and vascular smooth muscle cell proliferation. In humans, platelet-derived NO (PDNO) is also produced by the L-arginine/NO pathway through constitutive NOS. Platelet aggregation is inhibited by L-arginine, a precursor of NO, potentiated by NG-monomethyl-L-arginine, an inhibitor of NOS, and is accompanied by an increase in intracellular cyclic guanosine 3',5'-monophosphate. PDNO release during platelet aggregation is now recognized as a negative-feedback mechanism to inhibit not only platelet aggregation but also platelet recruitment.

Coronary risk factors are known to impair both EDNO10–12 and PDNO; however, there has not been a study measuring both EDNO and PDNO simultaneously in the same subjects, so it is still unknown whether impairment of these bioactivities coexist. Therefore, we investigated the relationship between EDNO and PDNO in patients with coronary risk factors.

Methods

Study Patients
The study group comprised 24 patients (17 men, 7 women; mean age, 67 years; range, 46–78 years) who underwent diagnostic cardiac catheterization and coronary arteriography. Femoral arterial endothelial function and PDNO release were studied in all patients. All cardiovascular medications were withheld for at least 72 h before the study. None of the patients had acute coronary syndromes, heart failure, or valvular heart disease. The study was approved by the institutional ethical committee, and written informed consent was obtained from all patients.

The coronary risk factors were hypertension (arterial blood pressure ≥140/90 mmHg), hypercholesterolemia (total serum cholesterol ≥220 mg/dl), current smoking (subjects who smoked ≥15 cigarettes per day for ≥5 years), diabetes mellitus (fasting glucose ≥126 mg/dl and/or plasma glucose ≥200 mg/dl 2 h after glucose administration), age (men ≥45 years old, women ≥55 years old or postmenopausal), and a family history of coronary artery disease. Family history was considered positive if a first-degree relative had clinical evidence of coronary artery disease at less than 55 years of age in male relatives or less than 65 years in female relatives.

Assessment of Femoral Arterial Function

After cardiac catheterization, femoral arterial endothelial function was assessed using a Doppler flow-wire technique as previously described. Briefly, a 5F angiographic catheter was introduced 1 cm beyond the end of a 6F femoral artery sheath and then a 0.018-in Doppler flow-wire (CardioMetrics Inc) was introduced through the catheter to 1 cm beyond the catheter tip to obtain an adequate flow velocity.
signal. Drugs were then infused through the sheath. A femoral angiogram was performed to exclude obstructive disease in the femoral circulation. The average peak velocity during each intervention was recorded. Because diameter measurements were not made at the level of the Doppler wire with each intervention, we calculated the femoral vascular resistance index (FVRI, mmHg·cm⁻¹·s⁻¹) as the mean arterial pressure divided by femoral blood flow velocity. To exclude any significant changes in femoral artery diameter at the site of the flow wire during conditions of increased blood flow, we measured femoral artery diameter at the site of the flow wire during administration of acetylcholine (ACh; 400 µg/min) and nitroglycerine (NTG; 200 µg/min) in 10 patients. There was no significant change in femoral artery diameter at the site of the flow wire during these drug infusions: baseline, 4.8±0.7 mm, ACh, 4.8±0.7 mm and NTG, 4.8±0.7 mm (all p = NS compared with baseline).

After the baseline measurements of flow velocity and mean arterial blood pressure, 2-min serial infusions of ACh at 50, 100, 200, and 400 µg/min were performed. After recovery and return to baseline values, NTG was serially administered at 50, 100, and 200 µg/min for 3 min. Peak flow velocity and arterial blood pressure were measured after each intervention.

**Preparation of Gel-Filtered Platelets**

Peripheral venous blood (20 ml) was drawn before examination of the FVRI, and gel-filtered platelets were prepared as described previously. Briefly, the citrated blood was centrifuged (150 G, 15 min, 22°C) and the platelet-rich plasma was separated and passed over a Sepharose-2B column equilibrated with HEPES-Tyrode's buffer without Ca²⁺. The platelet counts were finally adjusted to 2×10⁵ platelets/µL in Tyrode's solution.

**Measurements of PDNO With an NO-Specific Electrode**

We measured PDNO using an electronic NO meter (Model NO-501, Inter Medical Co) as described previously. The NO meter and electrodes were placed in an electromagnetic shield box to avoid electrical perturbation. The electrodes were placed in the chamber containing the gel-filtered platelets and after the addition of Ca²⁺ and fibrinogen, followed by ADP (5 µmol/ml), the working electrode was supplied with +0.6 V for electrochemical oxidation of NO. The change in the peak electrical current was considered the index of NO release.

**Statistical Analysis**

Data are expressed as mean±SD. The relationship between 2 parameters was analyzed by linear regression.

**Results**

**Clinical Characteristics (Table 1)**

The mean number of risk factors was 2.5±1.0, and the composite risk factor was 1 in 4 patients, 2 in 8, 3 in 8, and 4 in 4.

**Vascular Responses and Coronary Risk Factors**

ACh-mediated changes in the FVRI significantly correlated with the number of risk factors (r=–0.53, p<0.01; r=–0.54, p<0.01; r=–0.61, p<0.01; and r=–0.70, p<0.01 at 50, 100, 200, and 400 µg/min of ACh, respectively (Fig 1A), but the NTG-mediated changes did not (data not shown).

**PDNO Release and Coronary Risk Factors**

Mean PDNO was 14.5±6.5 pA (range 6–27 pA), and PDNO release negatively correlated with the number of risk factors (r=–0.55, p<0.01; Fig 1C).

**Relationship Between Vascular Responses and PDNO Release**

PDNO release significantly correlated with the ACh-mediated changes in FVRI at 50, 100, 200, and 400 µg/min of ACh (Fig 2). PDNO release did not correlate with the NTG-mediated changes (data not shown).

**Discussion**

ACh is commonly used as a probe for evaluating endothelial function, especially EDNO releasing capac-

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Table 1  Patients Characteristics

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>66±7</th>
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</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>17/7</td>
</tr>
<tr>
<td>Total cholesterol level (mg/dl)</td>
<td>192±32</td>
</tr>
<tr>
<td>LDL cholesterol level (mg/dl)</td>
<td>117±29</td>
</tr>
<tr>
<td>HDL cholesterol level (mg/dl)</td>
<td>50±14</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>96±12</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>11 (46)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>9 (38)</td>
</tr>
<tr>
<td>Previous smoking, n (%)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>Family history of coronary artery disease, n (%)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>
Endothelium-Derived and Platelet-Derived NO

impairment of PDNO release by human platelets, and we had a similar result in the human femoral circulation. This abnormality is usually attributed to decreased EDNO, which is synthesized from L-arginine via NOS in the endothelial cells. An NO-selective electrode is specifically capable of measuring real-time release of NO from aggregating platelets, and we have previously confirmed that the change in the electrical current obtained by the NO-selective electrode reflects the amount of NO released through the L-arginine/NO pathway in aggregating human platelets. Recently, we demonstrated that the number of coronary risk factors correlates with the degree of impaired ACh-mediated vasodilation in patients with coronary risk factors. PDNO release was correlated with ACh-mediated changes in the femoral vascular resistance index (FVRI).

First, the relatively small number of the subjects might have affected the results, but despite this, a significant correlation was found between EDNO and PDNO. Second, the contributions of endothelium-derived hyperpolarization factor and other vasodilating factors to ACh-induced vasodilation were not considered, but it has been demonstrated that the vasodilating effect of ACh is mainly caused by EDNO.

In conclusion, we have contributed to the understanding of the pathophysiology of atherothrombosis by demonstrating that both EDNO and PDNO bioactivities are impaired in patients with coronary risk factors, which suggests that both are involved in atherogenesis.

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References


