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CYP2C19 variants and epoxyeicosatrienoic acids in patients with microvascular angina

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ABSTRACT

Background: Categorization as a cytochrome P450 (CYP) 2C19 poor metabolizer (PM) is reported to be an independent risk factor for cardiovascular disease. Epoxyeicosatrienoic acids (EETs) are metabolites of arachidonic acid by CYP2C19 epoxygenases and anti-inflammatory properties, especially in microvascular tissues. We examined the impact of CYP2C19 polymorphisms and EETs on the patients with microvascular angina (MVA) caused by coronary microvascular dysfunction.

Methods and results: We examined CYP2C19 genotypes in patients with MVA ($n = 81$). MVA was defined as absence of coronary artery stenosis and epicardial spasms, and the presence of inversion of lactic acid levels between intracoronary and coronary sinuses in acetylcholine-provocation test or the adenosine-triphosphate-induced coronary flow reserve ratio was below 2.5. CYP2C19 PM have two loss-of-functon alleles (*2, *3). We measured serum dihydroxyeicosatrienoic acid (DHET) as representative EET metabolite.

In MVA, the patients with CYP2C19 PM were 34.6% and high sense C-reactive protein (hs-CRP) levels in CYP2C19 PM were significantly higher than that of non-PM group (0.165 ± 0.116 vs. 0.097 ± 0.113 mg/dL, $P = 0.026$). Moreover, DHET levels in CYP2C19 PM were significantly lower than that of non-PM (10.4 ± 4.58 vs. 15.6 ± 11.1 ng/mL, $P = 0.003$ (11,12-DHET); 12.1 ± 3.79 vs. 17.3 ± 6.49 ng/mL, $P = 0.019$ (14,15-DHET)).

Conclusions: The decline of EET owing to CYP2C19 variants may affects coronary microvascular dysfunction via chronic inflammation.

1. Introduction

Microvascular angina (MVA) is caused by coronary microvascular dysfunction, which can arise from chronic inflammation [1–3]. Epoxyeicosatrienoic acids (EETs) have been suggested to be anti-inflammatory [4] and potent vasodilators in the canine coronary microcirculation [5], raising the possibility that low levels of EETs may cause microvascular dysfunction in humans.

EETs are endothelium-derived hyperpolarizing factors (EDHFs) generated from metabolism of arachidonic acid by cytochrome P450 (CYP) 2C19 epoxygenases [6,7].

Endothelial hyperpolarization mediated by the opening of the potassium and calcium (KCa) channels is originally, recognized as the important initiating step for EDH mediated vasodilation [8]. Node et al. reported that EETs prevented leukocyte adhesion to the vascular wall by a mechanism involving inhibition of transcription factor, nuclear factor-κB and IκB kinase. The inhibitory effects of EETs were independent of their membrane-hyperpolarizing effects, suggesting that these molecules play an important non-vasodilatory role in vascular inflammation. Thus, EETs have been suggested to be anti-inflammatory properties [4]. Moreover, EETs act as potent vasodilators in canine coronary microcirculation [5]. It is associated that low levels of EETs may cause microvascular dysfunction via inflammation in humans.

Previous evidence has suggested that CYP enzymes not only play an important role in vascular tone, but are also associated with a higher risk of cardiovascular disease [9–13]. CYP2C19 is expressed in human endothelial cells, and enzymatic activity varies according to the number of CYP2C19 loss-of-function (LOF) alleles (*2, *3). CYP2C19 poor metabolizers (PM) have two LOF alleles (*2/*2, *2/*3, or *3/*3), and non-PM have none or one LOF allele (*1/*1, *1/*2, or *1/*3) [8,14].

Bertrand-Thiebault et al. reported that the blood levels of inflammatory markers (interleuikin-6 and hs-CRP) were significantly increased in CYP2C19 PM [15]. Furthermore, CYP2C19 variants are an independent risk factor for diabetic retinopathy [16] and coronary artery disease

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(CAD) [17] in humans. It is associated that the low levels of EETs with CYP2C19 variants cause increases in inflammatory markers, diabetic retinopathy, and CAD.

Thus, we examined the incidence and impact of CYP2C19 variants in MVA by measuring serum hs-CRP and dihydroxyeicosatrienoic acid (DHET) as representative EET metabolite in patients with MVA.

2. Methods

2.1. Study population

From April 2009 to January 2015, 1489 consecutive, hemodynamically and symptomatically stable patients with suspected angina were registered and underwent angiography in Kumamoto University Hospital. We excluded patients with possible heart failure (left ventricular ejection fraction <50%), previous diagnoses of CAD, left ventricular hypertrophy (defined as >12-mm left ventricular wall thickness in echocardiography), severe hypertension (HT) (>160/110 mm Hg), valvular heart disease, and malignant diseases. In addition, patients with elevated white blood cell counts (>9000) and/or serum hs-CRP (>0.5 mg/dL) were excluded to avoid the potentially confounding effects of occult infection or other systemic inflammatory diseases on hs-CRP levels.

After filtering, 1356 patients were enrolled in this study. Vasoactive drugs, including calcium-antagonists, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and nitrates were withdrawn at least 5 days before patients entered this study; sublingual nitroglycerin was withdrawn within 24-h before entering the study. Patients with HT were defined as >140/90 mm Hg. Patients with diabetes mellitus (DM) were defined as those with a 2-h glucose tolerance test ≥200 mg/dL, a fasting glucose level ≥126 mg/dL, an HbA1c score ≥6.5%, physician-diagnosed diabetes and/or the use of diabetic medication, and chronic kidney disease (CKD) testing with an estimated glomerular filtration rate <60 mL/(min · 1.73 m^2). Dyslipidemia (DLP) was defined as low-density lipoprotein >140 mg/dL, high-density lipoprotein <40 mg/dL, or triglyceride >150 mg/dL.

All procedures will be conducted in accordance with the Declaration of Helsinki and its amendments. The study protocol has been approved by the human ethics committee of each institution and written informed consent will be obtained from each patient or from the family of the patient.

2.2. Definition of MVA

All MVA cases experiencing chest pain and ischemic changes in the electrocardiogram (ECG) in the exercise stress test underwent cardiac catheterization. We defined the MVA was not only without epicardial spasm but also lactic acid levels between intracoronary and coronary sinuses in ACh-provocation test were increase, or adenosine triphosphate-induced coronary flow reserve ratio (ATP-CFR) was below 2.5 if lactic acid levels were decrease (Fig. 1) [2,18,19].

Acetylcholine-provocation test and measurement of the ATP-CFR ratio were conducted according to the Japanese Circulation Society guidelines [20]. Measuring ATP-CFR scores was also used to diagnose microvascular coronary dysfunction in non-obstructive CAD [21,22].

2.3. Intracoronary acetylcholine-provocation test

The method of intracoronary ACh-provocation test was described in detail previously [23–26]. In brief, incremental doses (20, 50, and 100 μg) of ACh chloride were injected into the left coronary artery over a period of 30 s each, and CAG was performed 1 min after the start of each provocation. The doses of ACh were administered at 5-min intervals.

Subsequently, 50 μg of ACh was injected into the right coronary artery without the FloWire™, followed by CAG. After the administration of intracoronary isosorbide mononitrate (ISDN) and CAG at an interval of 10 min, adenosine triphosphate (ATP; 150 μg/kg per minute) was administered via the central vein until maximal hyperemia was achieved for the calculation of ATP-CFR. ATP-CFR was calculated with the following formula: Hyperemia average peak velocity (APV)/Post-ISDN APV [27].

2.4. Angiographical identification of epicardial spasm

A positive finding for coronary spasm on CAG in the ACh test is defined as "transient, total, or sub-total occlusion (>90% stenosis) of a coronary artery." In addition, a definite diagnosis of vasospastic angina

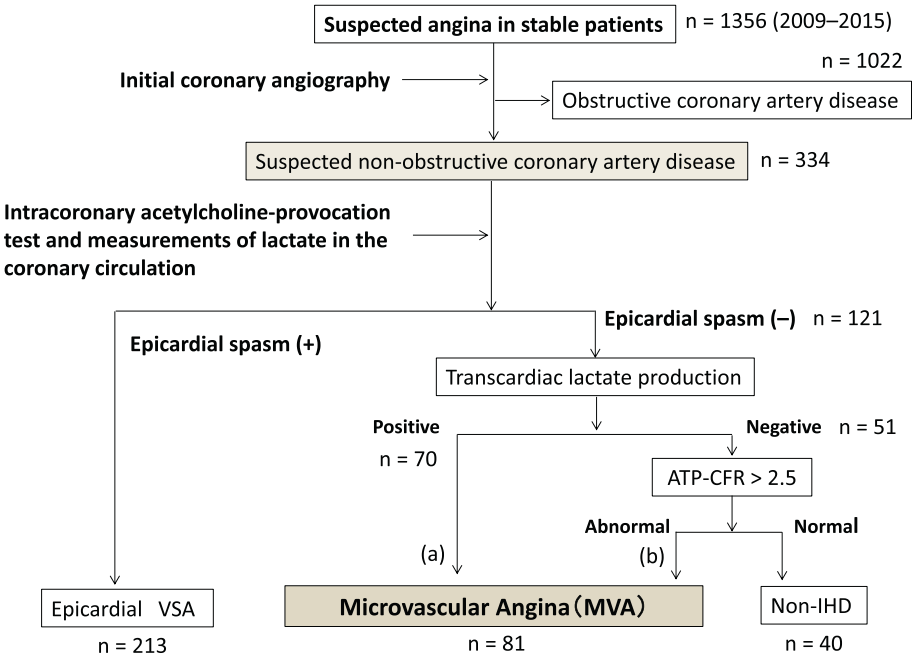

Fig. 1. Diagnostic flowchart. The definition of the patients with MVA is shown. ATP-CFR, adenosine triphosphate–induced coronary flow reserve; VSA, vasospastic angina; IHD, ischemic heart disease.