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Original article

Association between brachial-ankle pulse wave velocity and the ratio of L-arginine to asymmetric dimethylarginine in patients undergoing coronary angiography

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ABSTRACT

Background: Endothelial dysfunction causes vasomotor dysregulation and vascular stiffening in addition to structural changes. By influencing NO synthesis, deficiency of L-arginine relative to asymmetric dimethylarginine (ADMA), which is an L-arginine derivative that acts as a competitive NO synthase inhibitor, may lead to the promotion of arterial stiffness. This study investigated the relationship between the L-arginine/ADMA ratio and brachial-ankle pulse wave velocity (baPWV), an indicator of arterial stiffness.

Methods and results: This cross-sectional study enrolled 74 patients (62 men, 12 women; mean age, 67 ± 10 years) undergoing elective coronary angiography. A total of 54 (73%) patients had coronary artery disease. Serum L-arginine and ADMA were measured by high-performance liquid chromatography with fluorescence detection. The ratio of L-arginine to ADMA and the serum L-arginine level was associated with baPWV in univariate regression analysis (L-arginine/ADMA ratio: $\beta = -0.323$, $p = 0.005$; L-arginine: $\beta = -0.247$, $p = 0.034$). In addition, baPWV was related to blood hemoglobin concentration, hematocrit, brain natriuretic peptide level, symmetric dimethylarginine, renal function, blood pressure, and heart rate. In multivariate analysis, the L-arginine/ADMA ratio was a significant predictor of baPWV ($\beta = -0.310$, $p < 0.001$). In subgroup analyses, the L-arginine/ADMA ratio was associated with baPWV in elderly patients ($n = 46$, $\beta = -0.359$, $p = 0.004$), and in younger patients ($n = 28$, $\beta = -0.412$, $p = 0.006$).

Conclusion: A low L-arginine/ADMA ratio may be associated with high baPWV in patients undergoing coronary angiography.

Introduction

Arterial stiffness develops with aging, in part as a consequence of lifestyle-related diseases including hypertension, diabetes mellitus, renal dysfunction, and physical dysfunction, and the progression is associated with further cardiovascular events [1–3]. The primary cause of arterial stiffness is alteration of medial structural components by hemodynamic stress. Matrix metalloproteinases are involved in overproduction of abnormal collagen and degradation of elastin in the extracellular matrix [4]. Furthermore, the accumulation of glycation end products and calcium deposition contribute to the loss of vascular integrity and elasticity. Neurohormonal factors such as angiotensin II, insulin, and sympathetic nerve activation also have a role in the progression of vessel wall stiffness.

Endothelial dysfunction may promote vascular stiffening in addition to the structural changes [5,6], because it causes inappropriate vasoconstriction in peripheral arterioles leading to intima–media thickening [7]. Elevated peripheral arterial resistance promotes pulse wave reflection in the central arteries, and causes additional stress on the vessel wall. To maintain endothelial function in the large conductive arteries, nitric oxide (NO) plays a prominent role in vasodilatation, and inhibition of vascular smooth muscle cell proliferation, leukocyte adhesion, and platelet aggregation. L-Arginine serves as the sole source of nitrogen for NO, whereas asymmetric dimethylarginine (ADMA), which is an L-arginine derivative, competitively inhibits the

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activity of endogenous NO synthase (NOS). Protein arginine N-methyltransferase methylates arginine residues on proteins. ADMA and its stereoisomer symmetric dimethylarginine are generated from proteolysis of methylated nuclear proteins. Dimethylarginine dimethylaminohydrolase (DDAH) catalyzes the metabolism of ADMA to L-citrulline and dimethylamine.

Because ADMA competes with L-arginine for the NOS binding site, a decrease in L-arginine concentration relative to ADMA may decrease the ability to produce NO and increase arterial stiffness. Brachial-ankle pulse wave velocity (baPWV) is a feasible method to estimate arterial stiffness, and the results of a meta-analysis suggested that baPWV is able to predict cardiovascular events and mortality [8].

This study sought to determine the relationship between the L-arginine/ADMA ratio and baPWV in the patients undergoing coronary angiography. The different progression of arterial stiffness is a pivotal problem especially among elderly patients. Therefore, we also conducted subgroup analyses of the relation between L-arginine/ADMA ratio and baPWV by dividing the subjects at the age of 65 years.

Methods

Patients undergoing elective cardiac catheterization and baPWV and serum amino acid measurements from February 2011 to January 2012 at the National Defense Medical College (Tokorozawa, Japan) were enrolled in the study. Exclusion criteria included a low ankle brachial index (ABI) of less than 0.9 in both lower extremities, atrial fibrillation, and hemodialysis. The blood pressure and baPWV measurements were obtained using an FORM PWV/ABI automatic waveform analyzer (Omron Colin, Tokyo, Japan), equipped with electrocardiography, phonocardiography, and mechanocardiography functions. baPWV was recorded with patients in the supine position. Electrocardiogram electrodes were placed on both wrists. A microphone for detecting heart sounds was positioned on the left edge of the sternum. Cuffs with tonometric sensors were wrapped around both upper arms and ankles. The device measured the time intervals between pulse wavefronts for the right brachial artery and ankle arteries as pulse transit time (PTT), and automatically calculated baPWV using the following formula: baPWV = L/PTT (cm/s) (where L is the estimated brachial-ankle length). Coronary angiography was performed with a 4 Fr catheter system. Angiograms were taken from 4 standard projections for each right and left coronary artery. Coronary artery disease (CAD) was defined as the presence of coronary stenosis of >75% in at least 1 coronary vessel in the angiogram, a past history of myocardial infarction, or percutaneous or surgical coronary intervention.

Blood samples were drawn through a guiding sheath during coronary angiography before the administration of heparin, collected into plain tubes, and refrigerated immediately. Serum was obtained by centrifugation at 3000 rpm for 10 min at 4 °C. Derivatives of reactive oxidative metabolites were measured in serum using a free radical test (D-ROMS, Diacron International, Grosseto, Italy) within 12 h of blood collection. The D-ROMS test was used to quantify total hydroperoxide levels by measuring the ability of transition metals to catalyze the formation of free radicals. In this test, oxidized N,N-diethyl-para-phenylendiamine was detected spectrophotometrically at 505 nm [9,10].

Further serum samples were stored at −80 °C until required for amino acid analysis. Serum amino acids were measured by high-performance liquid chromatography on a SHIMADZU RF-20A instrument (SHIMADZU, Kyoto, Japan) with a Symmetry C18 column (3.9 mm × 150 mm; 5 μm particle size; Waters, Milford, MA, USA). The detection method used was based on fluorescent derivatization with AccQ-Fluor™ reagent (Waters) in accordance with previously described methods with several modifications [11,12].

The global arginine bioavailability ratio is an index to account for the proportion of arginine and its major catabolic products, defined as L-arginine/(L-ornithine + L-citrulline) [13]. This ratio was proposed to indicate the capacity to provide L-arginine for NO production.

Other risk factors of CAD were assessed using the following definitions. Hypertension was identified as blood pressure (BP) above 140/90 mmHg or receiving medication for the condition. Diabetes mellitus was diagnosed as fasting blood glucose >126 mg/dl or use of insulin or oral hypoglycemic agents. Hyperlipidemia was defined as total cholesterol >220 mg/dl, low-density lipoprotein cholesterol >140 mg/dl, or receiving anti-hyperlipidemic medication. Smoking index was defined as the number of cigarette-years smoked. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease equation modified for a Japanese population [14]. Ejection fraction was measured by echocardiography using Teichholz method or biplane modified Simpson method if appropriate.

Statistics

Summary data are presented as mean ± standard deviation (SD) with a 95% confidential interval (CI) for scales of normal distribution, or as median (interquartile ranges) for non-parametric variables. The comparison of variables in two groups was performed with Student's t-test or Mann–Whitney U-test. Cross-table analyses were performed using the Chi-squared test or Fisher's exact test if appropriate. Univariate and multivariate regression analyses were performed to identify independent variables from clinical features and laboratory parameters to explain baPWV. In the multivariate analysis, all the independent factors correlating with baPWV ($p < 0.1$) in the univariate analysis were included in a crude model (model 1). Next, a stepwise method was used to select effective explanatory variables from all of the variables used in model 1. Unnecessary variables were removed to obtain a good-fitting model (model 2). In model 2, L-arginine was not included for multicollinearity. All statistical analyses were performed using JMP version 10.0 (SAS Institute Inc., Cary, NC, USA). In all analyses, $p < 0.05$ was considered statistically significant.

Results

Patient characteristics and examinations

Nine patients with ABI <0.9 were excluded, leaving data from 74 patients for analysis. Of these patients, 54 (49 men, 5 women; mean age, 68 ± 8 years) had CAD and 20 (13 men, 7 women; mean age, 64 ± 13 years) did not have CAD. Among the patients with CAD, 21 had a history of percutaneous coronary intervention, three had a history of coronary bypass surgery, and 11 patients had prior myocardial infarction (Table 1). The numbers of patients with de novo or residual stenosis (0-, 1-, 2-, 3-vessels) at coronary angiography were 13, 13, 8, 12 in the subgroup of elderly patients (age ≥ 65 years, $n = 46$), whereas those were 11, 11, 4, 2 in the younger patients (age < 65 years, $n = 28$), respectively ($p = 0.194$). Thus, the prevalence of multi-vessel disease tended to be higher in the elderly group in this study (20/46 vs. 6/28 patients, $p = 0.054$).

Table 2 shows serum concentrations of amino acids and traditional coronary risk factors. Regarding physiological measurements, average baPWV was 1756 ± 358 cm/s, with systolic BP of 131 ± 19 mmHg and diastolic BP of 77 ± 11 mmHg. Heart rate (HR) was 68 ± 13 bpm. Ejection fraction measured by echocardiography was 66 ± 12%.