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Dimethylarginines, blood glucose, and C-reactive protein in patients with acute myocardial infarction

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SUMMARY

Background: Asymmetric dimethylarginine (ADMA), and its symmetrical stereoisomer (SDMA) — as methylated products of L-arginine, decrease nitric oxide (NO) availability. Their elevated levels in diabetes increase the risk of acute myocardial infarction (MI), through endothelial dysfunction.
Aims: We investigated the relationship between circulating levels of ADMA, SDMA and functional relevant parameters in patients with acute MI.
Methods: Prospective study from 31 MI patients hospitalized <12 h after symptom onset. Blood samples were taken on admission and serum levels of ADMA, SDMA and L-arginine were determined using high-performance liquid chromatography (HPLC).
Results: Mean age was 65y, most were male, hypertensive, 1/3rd were current smokers, or had a history of CAD and 23% were diabetic. ADMA and L-arginine values were similar whatever the risk factor, except for ADMA that was positively correlated with blood glucose ($r = 0.37$). In contrast, SDMA was correlated with age ($r = 0.43$), and admission glucose ($r = 0.57$). SDMA was elevated in hypertension, prior CAD, statin therapy and showed a trend toward an increase in diabetic patients ($p = 0.191$). Moreover, there was a trend toward an elevation of SDMA with decreased LVEF ($r = -0.25$). In multivariate linear regression analysis, blood glucose was an estimate of SDMA (ß = 0.373, $p = 0.025$), beyond traditional markers of dimethylarginines including kidney failure.
Conclusion: Our study showed that in patients with acute MI, SDMA, and only weakly ADMA, are associated with admission blood glucose, beyond traditional dimethylarginine determinants and may therefore have biological activity beyond renal function.

1. Introduction

L-Arginine (L-arg) is the precursor for the synthesis of nitric oxide (NO), a key signaling molecule via NO synthase (NOS). Reduced NO bioavailability may play an essential role in cardiovascular pathologies and metabolic diseases. L-arg deficiency syndromes in humans involve endothelial inflammation and immune dysfunctions [1]. The synthesis of NO is selectively inhibited by guanidino-substituted analogs of L-arg or methylarginines such as asymmetric dimethylarginine (ADMA), which results from protein degradation in cells. Many disease states, including cardiovascular diseases and diabetes, are associated with increased plasma levels of ADMA. Methylated arginines: ADMA and symmetric (SDMA) dimethyl-L-arginine are produced by the methylation of protein arginine residues, in the reaction catalyzed by arginine methyltransferases (PRMT). Most ADMA, but not SDMA, is degraded to citrulline and dimethylamine by dimethylarginine dimethylaminohydrolase (DDAH) wich is widely distributed throughout the organs and tissues, particularly in the liver and kidney. DDAH presents two isoforms: I and II; this way regulates ADMA levels and hence NO synthesis [2].

Unlike ADMA, which is a primary factor in the control of NOS activity, SDMA has insignificant inhibitory effects on the enzyme but might

Abbreviations: ACE inhibitor, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; ADMA, asymmetric dimethylarginine; BMI, body mass index; CAD, coronary artery disease; CK, creatine kinase; CRP, C-reactive protein; DBP, diastolic blood pressure; HR, heart rate; EC, endothelial cell; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; HPLC, high-performance liquid chromatography; ICU, intensive care unit; LDL-C, low-density lipoprotein-cholesterol; LVEF, left ventricular ejection fraction; NO, nitric oxide; NOS, NO synthase; SBP, systolic blood pressure; SDMA, symmetric dimethylarginine; STEMI, ST segment elevation myocardial infarction.

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inhibit NO-production through competition to arginine for transportation into the cell. SDMA is highly related to renal function [3], as urinary excretion is considered as the main route for elimination of SDMA, although it has been demonstrated in humans that the liver contributes in the elimination of SDMA [4].

Moreover, several lines of evidence have demonstrated that elevated ADMA levels are associated with endothelial cell (EC) dysfunction in healthy subjects and in patients with metabolic disease [5]. A positive relationship between long-term glycaemic control and plasma ADMA levels has been reported in patients with diabetes mellitus (DM), associated with micro- and macrovascular complications [6]. Moreover, a negative relationship has been reported between SDMA and insulin resistance [7]. SDMA, which is not a competitive inhibitor of NO synthase, failed to show any independent relationship with LDL-cholesterol. The significant link between ADMA and HDL-C level may be due to the modulation of eNOS activity [8]. Several studies reported that hyperglycemia induces modifications in HDLs, and LDLs functions, and is associated with inflammation and demonstrated that targeting inflammation improves glycemia, insulin resistance, and inflammatory profiles in patients at risk for development of cardiovascular and metabolic pathologies. It is well known that the intact endothelium may actively contribute to atherosclerotic disease initiation and/or progression. The underlying mechanisms leading to dysfunctional EC are not clear. Moreover, EC are heterogeneous in their response to physiopathological stimuli [9].

In patients with coronary artery disease (CAD), including acute myocardial infarction (MI), there is little information on the relationship between L-arg metabolism, NO bioavailability, glycemia and inflammation. Therefore, we aimed to examine the relation between plasma dimethylarginines, blood glucose, and a traditional marker of inflammation such as C-reactive protein (CRP) levels in patients hospitalized for acute MI.

2. Methods

2.1. Patients

The participants were recruited from the Observatoire des Infarctus de Côte d'Or (RICO) survey, a French regional survey of acute MI. Details on the survey have already been published [10]. In the present study, patients admitted to an intensive care unit between 4th May 2012 and 23rd January 2014 for acute MI within 12 h after symptom onset were included. MI was defined by an increase in serum troponin Ic [>upper limit of the hospital normal (ULN) range] associated with symptoms of ischemia and/or typical ECG signs [11]. ST-segment elevation MI (STEMI) was defined as chest pain lasting for ≥20 min with typical ECG changes including ≥1 mV ST segment elevation in two or more limb leads or ≥2 mV in two or more contiguous precordial leads. We collected data on patients' age, sex, cardiovascular risk factors (history of hypertension or treated hypertension, known history of diabetes, treated hypercholesterolaemia, body mass index (kg/m^2), current smoking (within three months)) and chronic treatments. Hemodynamic parameters on admission (heart rate, systolic and diastolic blood pressures), and Killip class >1 were also gathered. Left ventricular ejection fraction (LVEF) was measured by echocardiography 2 ± 1 day after admission using the Simpson method.

Blood samples for the assessment of biomarkers levels were collected on admission in EDTA containing tubes and stored at −80 °C until analysis.

The study was approved by the Consultative Committee of Protection of Persons in Biomedical Research of Burgundy and conducted in accordance with Declaration of Helsinki. All subjects gave their written consent to participate in the study.

2.2. Biological data

Blood samples were drawn on admission (Median time from symptom onset to blood sampling: 16 (8–30) hours). C-reactive protein (CRP), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations were measured on a Dimension analyzer (Dade Behring, Newark, NE). The level of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [12]. Plasma glucose concentrations (enzymatic method (glucose oxidase)) and creatinine levels were measured on a Vitros 950 analyzer (Ortho Clinical Diagnostics, Rochester, NY) and creatinine clearance was calculated with the Cockcroft formula. Glycated hemoglobin A1c (HbA1c) was measured with ion exchange HPLC (Bio-Rad Laboratories, Richmond, CA).

2.3. Dimethylarginines and L-arginine analysis

Samples were allowed to clot at room temperature for 30 min and centrifuged at 2500 rpm for 10 min at 4 °C. The serum was kept frozen at −80 °C until analysis. L-arginine, ADMA, and SDMA, were measured by high performance liquid chromatography (HPLC) [13]. Before the analysis, serum was added with N-monomethyl L-arginine (NMMA) as the internal standard and phosphate–buffered saline (PBS). This mixture was extracted on solid-phase extraction (SPE) cartridges (Phenomenex Strata X-C, Torrance, CA, USA). The cartridges were first conditioned with elution buffer (10/0.5/40/50; NH3 concentrated/1 M NaOH/bidistilled water/CH3OH; *v*/v/*v*/v) followed by bidistilled water before being loaded with the diluted sample. The SPE cartridge was consecutively washed with HCl (100 mmol/L) and methanol (1:1; v:v). ADMA and SDMA were eluted with elution buffer. All conditioning, washing and elution steps were achieved by vacuum suction. The eluate was dried under nitrogen, derivatized with ortho-phthaldialdehyde (OPA) reagent (1:1; v:v) and injected into the HPLC system, with a fluorescent detector Finingan Surveyor (Thermo Fisher (lexc: 340 nm, lem: 455 nm) and ChromolithH RP-18E column (10,064.6 mm) including a guard cartridge (1064.6 mm) supplied by Merck (Darmstadt, Germany). Chromatographic separation, at room temperature, was performed isocratically at 100% mobile phase A, with 25 mmol/L phosphate buffer (pH 6.8) containing 6.5% CH3CN, at a flow rate of 1.1 mL/min. After SDMA elution, mobile phase was switched to 100% mobile phase B, with ultrapure water: CH3CN (50:50, v:v), and the flow rate was increased to 3.0 mL/min to elute strongly retained compounds. Assays were performed in duplicate. The detection limits were 0.05 and 1.19 mmol/L and interday variabilities were 5.7 and 4.6% for ADMA and L-arginine, respectively [14].

2.4. Statistical analysis

Dichotomous variables are expressed as n (%) and continuous variables as mean ± SD or median (interquartile range). A Kolmogorov–Smirnov test was performed to assess the normality of continuous variables. Mann–Whitney test or Student's t test was used to compare continuous data, and the Chi 2 test or Fisher's test was used for dichotomous data. The threshold for significance was set at 5%. For continuous variables, Pearson or Spearman rank correlation analyses were performed, as appropriate. Multiple linear regression analysis was performed with SDMA as a dependent variable. Variables entered into the models were those with a significant relationship ($p < 0.05$) with the dependent variable in univariate analysis (i.e. hypertension, family history of CAD, age, creatinine clearance, and admission blood glucose). The threshold for significance was set at 5%. SPSS version 12.0.1 (IBM Inc., USA) was used for all of the statistical tests.

3. Results

Mean age was 65 years, most were male and hypertensive, almost 1/3rd were current smokers, or had a history of CAD (Table 1).