Correlation of the L-Arginine Pathway with Thrombo-Inflammation May Contribute to the Outcome of Acute Ischemic Stroke

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Background: Immune responses contribute to secondary injury after acute ischemic stroke (AIS), and metabolites of the L-arginine pathway are associated with stroke outcome. Here, we analyzed the relationship of the L-arginine pathway with thrombo-inflammatory biomarkers in AIS and their additive and independent associations to outcome. *Methods:* Serial changes in P-selectin, tPA, MCP-1, sCD40L, IL-6, IL-8, L-arginine, and asymmetric and symmetric dimethylarginine (ADMA, SDMA) were investigated in 55 patients with AIS and without infection within 6 and 72 hours after stroke onset. Outcomes were assessed as National Institutes of Health Stroke Scale (NIHSS) worsening by 24 hours, poststroke infection, and death by 1 month. *Results:* Serum levels of L-arginine showed negative correlation, whereas ADMA and SDMA showed positive correlation with thrombo-inflammatory biomarkers in the hyperacute phase. Most of these correlations disappeared by 72 poststroke hours. Correlation of MCP-1 with both ADMA and SDMA levels at 6 hours was associated with both NIHSS worsening and poststroke infections, respectively; sCD40L and SDMA correlation at 6 hours was also associated with NIHSS worsening. Negative correlation between P-selectin and L-arginine concentrations in the hyperacute phase was associated with NIHSS worsening. Strong negative correlation was found between IL-6 and L-arginine levels in the hyperacute phase in patients with poststroke infection. Only L-arginine and SDMA at 72 hours were independently associated with poststroke infection respectively. *Conclusions:* Concentration of L-arginine and ADMA/SDMA differentially correlates with thrombo-inflammation in the hyperacute phase of ischemic stroke. Such correlations are independently associated with poststroke infection but not with other outcomes. **Key Words:** L-arginine—dimethylarginine—cytokine—inflammation—ischemic stroke—outcome.

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Introduction

Thrombo-inflammatory molecules connecting coagulation with endothelial dysfunction and inflammation are key players in acute ischemic stroke.[1,2] Tissue plasminogen activator (tPA) influences blood–brain barrier permeability beside thrombolysis.[3] P-selectin recruits leukocytes, but also contributes to expression of major chemoattractants, such as monocyte chemotactic protein-1 (MCP-1) and IL-8.[4,5] CD40L is crucial in adaptive immune responses, but platelet-derived CD40L interacts with neutrophils and endothelial cells as well.[6,7] IL-6 produced by systemic immune cells and central nervous system (CNS) resident cells is elevated in both serum and cerebrospinal fluid after ischemic stroke.[8]

Nitric oxide (NO) plays a role in maintaining vascular integrity.[1] NO is synthetized by the oxidation of L-arginine, which can be inhibited by asymmetric dimethylarginine (ADMA).[2] ADMA increases the expression of inflammatory genes.[9] Symmetric dimethylarginine (SDMA) competes with arginine uptake and antagonizes the effects of L-arginine.[2,3]

The relationship between the L-arginine pathway and inflammation has been poorly explored in ischemic stroke.[10] We have previously reported that both thrombo-inflammatory molecules and the L-arginine pathway are associated with the outcome of ischemic stroke.[11,12] Therefore, we analyzed the relationship of the L-arginine pathway with thrombo-inflammatory biomarkers in acute ischemic stroke and their additive or independent association with outcomes.

Materials and Methods

The study was approved by the Local Ethics Committee of the University of Pecs.

Subjects

Fifty-five patients (mean age 70 ± 10 years, male 51%, female 49%) were enrolled within 6 hours after onset of ischemic stroke at the Department of Neurology, University of Pecs. Twenty-two percent of the patients were smokers, 14% had coronary artery disease, 72% had hypertension, and 13% had diabetes. Hemorrhagic stroke, infection, and/or fever less than 4 weeks were exclusion criteria. Severity of stroke was measured by the National Institutes of Health Stroke Scale (NIHSS) on admission and 24 hours later.

Infections

An evidence-based guideline was followed to detect infectious complications (in short, physical and laboratory measures including white blood count (WBC), erytrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hsCRP), procalcitonin, fever, abnormal urine, chest X-ray, or positive cultures).[13] NIHSS worsening by 24 hours and poststroke infections as outcomes were evaluated if it occurred from the third poststroke day until discharge from hospital or death.

Biomarkers

Blood samples were taken on admission (within 6 hours after onset) and 72 hours later.[11,12] Concentration of P-selectin, MCP-1, CD40L, IL-6, IL-8, and tPA was measured by immunoassay (BMS711F, Bender GmbH, Campus Vienna Biocenter 2, Vienna, Austria). L-arginine, ADMA, and SDMA were measured in the sera by high-performance liquid chromatography as described previously.[14]

Statistical Analysis

Statistical calculation was performed using the SPSS (Windows, Version 16.0. SPSS Inc, Chicago, Illinois, USA) 11.0 package. Spearman's correlation was used to analyze the data of the total population and different subgroups based on NIHSS worsening, poststroke infection, and death. A multiple regression analysis was also performed to find independent variables. As 9 patients were treated with intravenous recombinant tPA (rtPA), these patients received additional L-Arginine, which is contained in the available solution formula. For statistical analysis of L-Arginine levels, these patients were analyzed in a subgroup. Data were presented as correlation coefficients. A P value <.05 was regarded as significant.

Results

Correlation between the L-Arginine Pathway and Thrombo-Inflammatory Biomarkers

Concentration of biomarkers within 6 hours after stroke and 72 hours later is shown in Table 1.

First, we examined the correlation between the L-arginine pathway and thrombo-inflammatory biomarkers within 6 hours after the onset of stroke, and 72 hours later. In general, the serum levels of L-arginine showed negative correlation, whereas ADMA and SDMA showed positive correlation with thrombo-inflammatory biomarkers (Table 2). Such correlations were more characteristic in the hyperacute phase.

Within Six Hours

The serum levels of L-arginine showed negative correlation with concentrations of IL-6, IL-8, and tPA. Both ADMA and SDMA levels correlated with MCP-1. The concentration of SDMA correlated with P-selectin levels. The ratio of both L-arginine/ADMA and L-arginine/SDMA negatively correlated with concentrations of all thrombo-inflammatory biomarkers except P-selectin; sCD40L levels correlated only with L-arginine/SDMA ratio. The concentration of tPA showed a negative correlation with L-arginine, and L-arginine/ADMA and L-arginine/SDMA ratios, respectively (Table 2).