Metabolic Profiling of Arginine and Nitric Oxide Pathways Predicts Hemodynamic Abnormalities and Mortality in Patients With Cardiogenic Shock After Acute Myocardial Infarction

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Background—It is unclear whether abnormalities of arginine and nitric oxide metabolism are related to hemodynamic dysfunction and mortality in patients with cardiogenic shock (CS) after acute myocardial infarction.

Methods and Results—Plasma metabolites reflecting arginine bioavailability, nitric oxide metabolism, and protein oxidation were analyzed by mass spectrometry in patients with CS (n=79) and age- and gender-matched patients with coronary artery disease and normal left ventricular function (n=79). CS patients had higher levels of asymmetric dimethylarginine (ADMA; P<0.0001), symmetric dimethylarginine (P<0.0001), monomethylarginine (P=0.0003), nitrotyrosine (P<0.0001), and bromotyrosine (P<0.0001) and lower levels of arginine (P<0.0001), ratio of arginine to ornithine (P=0.03), and ratio of arginine to ornithine plus citrulline (P=0.0003). CS patients with elevated ADMA levels were 3.5-fold (95% confidence interval, 1.4 to 11.3; P=0.02) more likely to die in 30 days than patients with low ADMA levels. ADMA remained the only independent predictor of mortality on multiple logistic regression analysis. In patients with normal renal function, symmetric dimethylarginine levels inversely correlated with mean arterial pressure and systemic vascular resistance, whereas levels of ADMA correlated with pulmonary capillary wedge pressure and both systolic and diastolic pulmonary artery pressures. Despite dramatic elevations, levels of protein oxidation products did not predict hemodynamic dysfunction or mortality in CS patients.

Conclusions—CS is characterized by an arginine-deficient and highly specific pro-oxidant state, with elevated levels of methylated arginine derivatives, including endogenous nitric oxide synthase inhibitors. Levels of methylated arginine derivatives strongly correlate with hemodynamic dysfunction. Among all clinical and laboratory parameters monitored, ADMA levels were the strongest independent predictor of 30-day mortality. (Circulation. 2007;116:2315-2324.)

Key Words: myocardial infarction ■ nitric oxide ■ oxidative stress ■ shock

A cute myocardial infarction (MI) complicated by cardiogenic shock (CS) is associated with substantial morbidity and mortality.1 Mortality rates remain high despite early revascularization.2-3 Specific molecular pathways that contribute to the pathological features of CS and its negative clinical consequences have not been identified. Understanding these processes may provide targets for pharmacological manipulation and identify systemic biomarkers that predict clinical outcome.

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Several lines of evidence suggest that excess nitric oxide (NO) may play a role in the pathogenesis of CS.4 Constitutive expression of the endothelial isoform of NO synthase (NOS) produces low and protective levels of NO. Higher levels of NO are produced by an inducible isoform of NOS (iNOS) expressed by many cell types in response to inflammatory stimuli.5 Because myocardial injury stimulates the expression of proinflammatory cytokines, iNOS expression and generation of potentially toxic levels of NO and its reactive oxidant derivative, peroxynitrite, are thought to occur in CS complicating acute MI.6 Consistent with that view, NO levels within the coronary arteries are increased in the setting of acute experimental heart failure.6 Increasing concentrations of NO potentially contribute to the hypotension and diminished cardiac output of CS by depressing myocardial contractil-
ty,7,8 causing vascular relaxation4 and blocking responsivity to endogenous catecholamines.9 Administration of NOS inhibitors under some circumstances is reported to promote an increase in contractility and systemic afterload in animals10 and humans.11 Some preliminary clinical studies suggest that these hemodynamic effects may increase survival and decrease the need for support with intra-aortic balloon pumps and mechanical ventilation.4,12,13

Despite evidence that NOS and NO play a role in CS, little is known about the levels of the NOS substrate arginine and its metabolites. Arginine availability may limit NO synthesis, particularly in cells expressing iNOS. Arginine can be depleted by both NOS14 and arginase15 activity. In addition, some endogenously formed methylated derivatives of arginine are NOS inhibitors. Monomethylarginine (MMA), symmetric dimethylarginine (SDMA), and asymmetric dimethylarginine (ADMA) are formed by posttranslational methylation of arginine residues of proteins and released as free amino acids after protein degradation.16 ADMA and MMA inhibit NOS.16

No studies have simultaneously quantified a broad array of metabolites involved in NO synthesis and its regulation in the setting of CS. We hypothesized that such determinations, in combination with monitoring multiple molecular markers of distinct oxidative pathways, including NO-derived oxidants, would provide insights into the arginine/NO “metabolome” and its relation to hemodynamic parameters and cardiovascular risks. We developed a stable isotope dilution assay using liquid chromatography with online electrosporo ionization tandem mass spectrometry (LC/ESI/MS/MS) for the simultaneous quantification of a panel of plasma metabolites reflecting arginine bioavailability and NO metabolism. In the present studies, we evaluated plasma samples obtained from patients with CS after acute MI and age- and gender-matched subjects with stable coronary artery disease (CAD) and normal left ventricular function.

**Methods**

**CS Subjects**

Baseline samples were evaluated from 79 subjects with acute MI complicated by CS participating in the Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock (SHOCK-2) trial, an assessment of efficacy of the NOS inhibitor Nω-monomethyl-L-arginine (L-MMA).17,18 Plasma samples and hemodynamic measurements were obtained after diagnosis of CS but before any trial-related interventions. Informed consent was obtained from all participants or authorized surrogates. CS was defined as the combination of (1) peripheral signs of tissue hypoperfusion (decreased urine output, cool extremities), (2) pulmonary capillary wedge pressure ≥15 mm Hg, (3) systolic blood pressure <100 mm Hg on vasopressor therapy, and (4) cardiac index <2.2 L · min⁻¹ · m⁻² that persisted 1 to 12 hours after demonstration of a patent infarct-related artery, typically after percutaneous coronary intervention, in the setting of an acute MI. Hemodynamic parameters were determined by a Swan-Ganz catheter placed for clinical indications before administration of the study drug. Creatinine clearance was estimated by the Modification of Diet in Renal Disease formula.

**Stable CAD Subjects**

Plasma samples of 79 age- and gender-matched subjects with stable angina pectoris, angiographic CAD, and intact left ventricular systolic function (ejection fraction ≥50%) were randomly obtained from the GeneBank study, a repository of consecutive patients who presented for diagnostic coronary angiography for a clinical indication at the Cleveland Clinic. The Institutional Review Board at the Cleveland Clinic Foundation approved all study protocols.

**MS Analysis of NO Metabolome and Protein Oxidation Products**

Plasma samples were stored at −80°C and subsequently thawed at room temperature before analysis. Aliquots (100 µL) of plasma were combined with 100 µL of 10 mmol/L [13C6]arginine in water (internal standard) and mixed by vortexing. Proteins were precipitated by the addition of 550 µL acetonitrile and centrifugation. Acetonitrile and water were removed from supernatants under vacuum; the residue was resuspended in 50% (vol/vol) methanol/water; and levels of amino acids were quantified by LC/ESI/MS/MS analysis with an ABI 365 triple quadrupole mass spectrometer (Applied Biosystems Inc, Foster City, Calif) equipped with Ionics EPI 10+ upgrade (Concord, Ontario, Canada) and an ESI needle connected to an Aria LX4 series multiplexed high-performance LC system with Flux pumps (Cohesive Technologies, Franklin, Mass). Amino acids were separated on a 250×4.6 mm Rsexochrom S5–100-P phenyl column (Regis Chemical, Morton Grove, Ill) and resolved using a discontinuous gradient with 0.2% formic acid (solvent A), 10 mmol/L ammonium formate in methanol (solvent B), and 10 mmol/L ammonium formate in water (solvent C). The gradient used was as follows: The column was first equilibrated with 100% solvent A at 800 µL/min and held at this composition for 0.5 minutes after the injection; a linear gradient was then run to 25% solvent B and 25% solvent C (50% solvent A) over the next 3 minutes and held for 8 minutes at a flow rate of 800 µL/min. At 11.5 minutes, the flow rate was increased to 1000 µL/min, and the solvent composition was changed to 100% solvent B in a linear fashion over 2.5 minutes, held at 100% solvent B for 3 minutes, and then changed to 100% solvent C at 100 µL/min for 3 minutes. MS analyses were performed online using LC/ESI/MS/MS in the positive ion mode with multiple reaction monitoring using unique characteristic parent–daughter ion transitions for each analyte. Cone potentials and collision energy were optimized for each analyte, and standard curves were generated with [13C6]arginine as internal standard. Each analyte monitored demonstrated nearly quantitative recovery, good linearity over multiple orders of magnitude in the concentration range, and intra-assay and interassay coefficients of variance of <10%. The coefficients of variance for arginine and related metabolites typically range from 2% to 15% for duplicate preparations/injections. The lower limit of quantification for MMA was 0.04 µmol/L, and ranged from 0.1 to 0.3 µmol/L for the other analytes. Levels of protein oxidation products were measured with stable isotope dilution LC/ESI/MS/MS using the ABI 365 with Ionics upgrade.19–21

**Statistical Analysis**

All statistical analyses were performed with JMP version 5.1.2 (SAS, Cary, NC). Results are expressed as median (interquartile range). Comparisons of levels of NO-related amino acid and oxidized amino acid levels between patients with CS and patients with CAD and normal left ventricular function were performed with the Mann–Whitney test. Spearman correlations determined the relationship between the levels of amino acids and both hemodynamic indexes and creatinine clearance. We used χ² analysis to compare mortality rates between groups with biomarkers above and below the median. Continuous variables that correlated significantly with mortality were studied by multiple logistic regression analysis to determine whether any of these factors remained independent predictors. Variables that were clearly not significant on regression analysis (age) or correlated significantly with ADMA levels (body mass index) were not included in this analysis. A 2-sided value of P<0.05 was considered significant.
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Patient Characteristics

The baseline demographics of the patients with CS and stable CAD with intact left ventricular function (median age, 72 years; 67% male) are summarized in Table 1. Patients with CS had a lower body mass index and left ventricular ejection fraction. Hemodynamic analysis revealed decreased mean arterial pressure and cardiac index in association with elevated pulmonary artery and capillary wedge pressures. There was a high incidence of renal dysfunction, with 47% of patients having a high plasma creatinine and low estimated creatinine clearance.

Table 1. Clinical Characteristics of Subjects With Cardiogenic Shock

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CS Patients (n=79)</th>
<th>CAD Without CS (n=79)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.4 (24.1–30.1)</td>
<td>29.4 (26.3–35.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>25.0 (15.0–35.0)</td>
<td>60.0 (55.0–65.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma creatinine, mg/dL</td>
<td>1.30 (1.00–1.80)</td>
<td>1.0 (0.8–1.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estimated creatinine clearance, mL/min</td>
<td>55.4 (37.7–76.7)</td>
<td>79.0 (56.0–107.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>88.5 (79.5–99.5)</td>
<td>139.0 (123.0–155.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>50.0 (39.0–56.0)</td>
<td>74.0 (65.0–83.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>75.0 (65.0–82.0)</td>
<td>96.7 (86.3–105.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure, mm Hg</td>
<td>20.0 (16.0–24.0)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Cardiac index, L · min⁻¹ · m⁻²</td>
<td>1.73 (1.32–2.11)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Systolic pulmonary artery pressure, mm Hg</td>
<td>37.0 (31.0–43.0)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Diastolic pulmonary artery pressure, mm Hg</td>
<td>21.0 (17.0–25.0)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, dyne/cm²</td>
<td>163.3 (86.3–266.7)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Systemic vascular resistance, dyne/cm²</td>
<td>1419 (1099–1763)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Hemodynamic parameters for CS patients were recorded while subjects received circulatory support with vasopressors and intra-aortic balloon pumps. Results are expressed as median (interquartile range) for continuous variables and as percentage for categorical variables.

NO-Related Plasma Amino Acids and Protein Oxidation Products in CS and CAD Subjects

The levels of an array of amino acids influencing NO synthesis were examined in patients with CS and compared with age- and gender-matched patients with stable CAD and normal left ventricular function (Table 2). CS was characterized by an arginine-deficient state and elevated levels of methylated arginine derivatives. CS patients had lower plasma levels of arginine (30.8 versus 62.0 μmol/L; P<0.0001), ratio of arginine to ornithine (0.91 versus 1.30; P=0.03), and ratio of arginine to ornithine plus citrulline (0.32 versus 0.86; P<0.0003). The last 2 values reflect the ratio of arginine to its metabolites and are thought to serve as better global indexes of arginine deficiency. Subjects with CS had higher levels of the endogenous NOS inhibitors ADMA (1.25 versus 0.71 μmol/L; P<0.0001) and MMA (0.10 versus 0.08 μmol/L; P=0.0003) and the noninhibiting methylated analogue SDMA (1.13 versus 0.33 μmol/L; P<0.0001). Accordingly, lower ratios of arginine to ADMA (21.8 versus 82.7; P<0.0001), arginine to SDMA (25.4 versus 172.5; P<0.0001), and arginine to MMA (324.6 versus 845.8; P<0.0001) also were found in patients with CS.

Molecular Markers of Distinct Protein Oxidation Pathways in CS

Levels of molecular markers of systemic oxidative stress caused by distinct oxidative pathways also were investigated in patients with CS compared with patients with stable CAD with intact left ventricular systolic function (Table 2). Patients with CS had higher levels of nitrotyrosine (26.5 versus 12.8 μmol/mol tyrosine; P<0.0001), bromotyrosine (131.6 versus 11.7 μmol/mol tyrosine; P<0.0001), orthotyrosine (346.3 versus 86.8 μmol/mol tyrosine; P<0.0001), metatyrosine (260.6 versus 32.5 μmol/mol tyrosine; P<0.0001), and dityrosine (415.0 versus 153.4 μmol/mol tyrosine; P<0.0001).

Impact of Renal Function on NO and Arginine-Related Biomarkers in CS

Given the high incidence of renal dysfunction in the CS patients and the potential for renal clearance to serve as an important determinant of plasma levels of amino acids, levels of the monitored NO-related arginine metabolites and protein oxidation products were stratified according to renal function (Table 3). Compared with CS patients with normal renal function, CS patients with abnormal renal function (plasma creatinine >1.4 mg/dL) had higher systemic levels of SDMA (1.33 versus 0.91 μmol/L; P=0.0004), ADMA (1.29 versus 1.09 μmol/L; P=0.08), and homocitrulline (0.53 versus 0.26 μmol/L; P=0.0007) and lower ratios of arginine to SDMA (20.6 versus 27.3; P=0.05) and arginine to ADMA (19.5 versus 22.9; P=0.09). These differences are consistent with the known renal clearance of SDMA and the significant role of the kidney in the metabolism of ADMA. In a complementary fashion, significant correlations were observed between creatinine clearance and SDMA (r = −0.43, P=0.0002), citrulline (r = −0.27, P=0.03), homocitrulline (r = −0.40,
Apart from a trend toward lower levels of chlorotyrosine with abnormal renal function, there were no differences in levels of the molecular markers of oxidative stress in CS patients with normal or abnormal renal function.

### Correlation With Hemodynamic Abnormalities

Systemic levels of NO-related arginine metabolites in CS patients were evaluated with regard to their correlation with hemodynamic abnormalities (Table 4). Hemodynamic parameters were measured in the setting of vasopressor therapy in all CS patients, most of whom also received circulatory...
support with an intra-aortic balloon pump. CS patients were stratified as having normal or abnormal renal function on the basis of systemic creatinine levels. In patients with CS and normal renal function, SDMA levels were inversely correlated with mean arterial pressure ($r = -0.56, P = 0.001$) and systemic vascular resistance ($r = -0.45, P = 0.02$), whereas ADMA levels correlated with pulmonary capillary wedge pressure ($r = 0.45, P = 0.02$) and systolic pulmonary artery pressure ($r = 0.37, P = 0.04$). In CS patients with abnormal renal function, systemic levels of SDMA and ADMA did not correlate with any hemodynamic parameter. Remarkably, despite the substantial elevations noted in many of the molecular markers of oxidative stress that reflect several distinct oxidative pathways, no significant correlations were observed between any species of oxidized amino acid and any hemodynamic parameter, regardless of the state of renal function (data not shown).

**Correlation of ADMA Levels With Mortality**

CS patients had an overall high 30-day mortality rate (39.2%). As was recently reported, mortality rates among the different arms of the SHOCK-2 trial did not demonstrate significant differences in mortality rates, ranging from 35% in the placebo arm to 53% in patients receiving 0.15- or 0.5-mg/kg doses of MMA and 28% in patients receiving 1.0- or 1.5-mg/kg doses of L-MMA. Baseline levels of the array of NO-related arginine metabolites were evaluated to see whether any correlated with 30-day mortality and thus could serve as potential predictors of mortality (Table 5). Levels of ADMA were higher in patients who subsequently died compared with those who survived (1.40 versus 1.18 $\mu$mol/L, respectively; $P = 0.03$). Patients who died demonstrated a trend toward higher levels of SDMA (1.26 versus 1.05 $\mu$mol/L; $P = 0.08$). In univariate analyses, CS patients with elevated ADMA levels (above the median) were 3.54-fold

### Table 4. Spearman Correlation of Systemic Levels of the Methylated Arginine Derivatives SDMA and ADMA and the Arginine/Ornithine Ratio With Hemodynamic Parameters in Patients With CS Stratified According to Presence or Absence of Renal Dysfunction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SDMA</th>
<th>ADMA</th>
<th>Arg/Orn</th>
<th>SDMA</th>
<th>ADMA</th>
<th>Arg/Orn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure</td>
<td>$-0.56$</td>
<td>$0.001$</td>
<td>$-0.35$</td>
<td>$0.06$</td>
<td>$0.77$</td>
<td>$-0.32$</td>
</tr>
<tr>
<td>Cardiac index</td>
<td>$-0.45$</td>
<td>$0.02$</td>
<td>$-0.16$</td>
<td>$0.44$</td>
<td>$0.43$</td>
<td></td>
</tr>
<tr>
<td>Systemic vascular resistance</td>
<td>$0.28$</td>
<td>$0.15$</td>
<td>$0.45$</td>
<td>$0.02$</td>
<td>$0.18$</td>
<td></td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure</td>
<td>$0.28$</td>
<td>$0.31$</td>
<td>$0.37$</td>
<td>$0.04$</td>
<td>$0.09$</td>
<td></td>
</tr>
<tr>
<td>Systolic pulmonary artery pressure</td>
<td>$0.11$</td>
<td>$0.58$</td>
<td>$0.34$</td>
<td>$0.07$</td>
<td>$0.09$</td>
<td></td>
</tr>
<tr>
<td>Diastolic pulmonary artery pressure</td>
<td>$-0.13$</td>
<td>$0.57$</td>
<td>$0.18$</td>
<td>$0.45$</td>
<td>$-0.14$</td>
<td>$0.50$</td>
</tr>
</tbody>
</table>

Arg indicates arginine; orn, ornithine.

### Table 5. Systemic Levels of Arginine and Its Methylated Derivatives in Patients With CS Stratified According to 30-Day Mortality Rates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dead (n=31)</th>
<th>Alive (n=48)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine, $\mu$mol/L</td>
<td>31.1 (23.4–47.9)</td>
<td>29.5 (19.9–40.3)</td>
<td>0.38</td>
</tr>
<tr>
<td>ADMA, $\mu$mol/L</td>
<td>1.40 (1.02–1.89)</td>
<td>1.18 (0.75–1.45)</td>
<td>0.025</td>
</tr>
<tr>
<td>SDMA, $\mu$mol/L</td>
<td>1.26 (0.96–1.53)</td>
<td>1.05 (0.70–1.31)</td>
<td>0.08</td>
</tr>
<tr>
<td>MMA, $\mu$mol/L</td>
<td>0.12 (0.08–0.16)</td>
<td>0.09 (0.06–0.13)</td>
<td>0.66</td>
</tr>
<tr>
<td>Arginine/ADMA</td>
<td>19.5 (15.4–27.4)</td>
<td>22.4 (15.6–32.5)</td>
<td>0.33</td>
</tr>
<tr>
<td>Arginine/SDMA</td>
<td>25.3 (16.2–29.1)</td>
<td>26.2 (16.3–40.2)</td>
<td>0.31</td>
</tr>
<tr>
<td>Arginine/MMA</td>
<td>330.3 (190.4–385.6)</td>
<td>318.9 (178.6–425.3)</td>
<td>0.58</td>
</tr>
<tr>
<td>Arginine/ornithine</td>
<td>0.95 (0.43–1.52)</td>
<td>0.87 (0.46–1.64)</td>
<td>0.70</td>
</tr>
<tr>
<td>Arginine/ornithine + citrulline</td>
<td>0.35 (0.17–0.45)</td>
<td>0.25 (0.17–1.03)</td>
<td>0.67</td>
</tr>
<tr>
<td>Nitrotyrosine, $\mu$mol/mol tyrosine</td>
<td>25.3 (14.3–37.9)</td>
<td>29.8 (17.9–41.2)</td>
<td>0.39</td>
</tr>
<tr>
<td>Chlorotyrosine, $\mu$mol/mol tyrosine</td>
<td>5.1 (1.3–11.6)</td>
<td>5.2 (2.5–12.9)</td>
<td>0.58</td>
</tr>
<tr>
<td>Bromotyrosine, $\mu$mol/mol tyrosine</td>
<td>138.3 (107.9–180.7)</td>
<td>125.3 (95.3–165.9)</td>
<td>0.23</td>
</tr>
<tr>
<td>Orthotyrosine, $\mu$mol/mol tyrosine</td>
<td>290.4 (220.2–389.4)</td>
<td>354.0 (283.4–423.3)</td>
<td>0.08</td>
</tr>
<tr>
<td>Metatyrosine, $\mu$mol/mol tyrosine</td>
<td>236.1 (163.2–386.9)</td>
<td>277.6 (210.1–400.6)</td>
<td>0.25</td>
</tr>
<tr>
<td>Dityrosine, $\mu$mol/mol tyrosine</td>
<td>451.0 (306.7–563.0)</td>
<td>406.7 (303.8–536.9)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Results are expressed as median (interquartile range).
(95% CI, 1.36 to 11.29; P = 0.02) more likely to die over the ensuing 30 days than patients with low ADMA levels (the Figure and Tables 5 and 6). Interestingly, no oxidized amino acid species predicted mortality in patients with CS, including levels of nitrotyrosine, a molecular marker of NO-derived oxidants. Multiple logistic regression analysis revealed that ADMA was the only factor that remained an independent predictor of mortality (odds ratio [OR], 3.19; 95% confidence interval [CI], 1.02 to 12.81; P < 0.05). Moreover, multivariate analyses aimed at assessing the potential influence of the placebo versus L-MMA treatment arm (individual dose or combinations of doses) on mortality revealed little impact of treatment, with ADMA remaining an independent predictor of 30-day mortality (OR, 5.56 to 5.90, depending on model; P < 0.05 for all models). Furthermore, ADMA levels at baseline had no impact on predicting the clinical efficacy of the L-MMA treatment arms (data not shown).

Discussion

Reports suggest that activation of the NO synthetic pathway contributes to the pathophysiology of CS and that administration of NOS inhibitors may be therapeutic.4,12,13 The present analysis elucidated the role that perturbations of the NO synthetic pathway may play in CS patients, with induction of both arginase and iNOS, may contribute to the high mortality observed. Of CS, with induction of both arginase and iNOS, the presence of a systemic inflammatory syndrome in the setting of CS, with induction of both arginase and iNOS, may contribute to the high mortality observed.

Table 6. ORs (95% CIs) for Predictors of Mortality in Patients With CS Based on Univariate Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR (95% CI), Univariate</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.08 (1.03–1.15)</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI</td>
<td>0.86 (0.76–0.96)</td>
<td>0.02</td>
</tr>
<tr>
<td>Estimated Cr Clear</td>
<td>0.96 (0.93–0.98)</td>
<td>0.0009</td>
</tr>
<tr>
<td>SBP</td>
<td>0.96 (0.93–0.99)</td>
<td>0.047</td>
</tr>
<tr>
<td>ADMA</td>
<td>3.54 (1.36–11.29)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; Cr Clear, creatinine clearance; and SBP, systolic blood pressure.

These results extend our understanding of the cardiovascular effects of NO. The endothelial isoform of NOS produces low levels of NO that play beneficial roles in the maintenance of vascular homeostasis.23 NO bioavailability is decreased in subjects with atherosclerotic risk factors, endothelial dysfunction, and CAD.24 Those observations suggested that administration of the NOS substrate arginine might increase NO synthesis and be of potential benefit in cardiovascular disorders. However, although administration of L-arginine has been demonstrated to improve endothelial function in hypercholesterolemic subjects25 and to treat hypertension,26 recent randomized trials showed increased mortality with arginine supplementation after MI.27 Nonetheless, the present findings show that patients with CS after an MI are in a state of relative arginine deficiency. The cause of this deficiency remains unclear.

Increased conversion of arginine to ornithine and citrulline may play an important role in CS patients. The present results imply that both NOS, which converts arginine to citrulline, and arginase, which converts arginine to ornithine, may be elevated in CS patients. The implication of higher arginase activity in CS is consistent with reports of arginase induction by inflammatory cytokines known to be elevated in cardiac ischemia-reperfusion injury and CS28 and extends reports of increased arginase activity in a number of disease states, including MI,29 asthma,30 breast cancer,31 and pulmonary hypertension.22,32

NO also can be produced in large and potentially toxic amounts by iNOS expressed in response to trauma and inflammatory stimuli.4 MI and CS result in elaboration of proinflammatory cytokines likely to result in an increased expression of iNOS.4 High levels of iNOS and NO have been described in the experimental setting of ischemia-reperfusion.33 Intracardiac and intravascular synthesis of high levels of NO, superoxide, and peroxynitrite, a potent and highly reactive oxidant formed by spontaneous reaction of NO and superoxide, may exert deleterious effects on myocardial function and systemic hemodynamics in CS. NO is a myocardial depressant,7,8 a vasorelaxant,9 and an antagonist of the vasoconstrictive effect of catecholamines,9 including those produced endogenously in response to CS. Together, these effects would be expected to reduce cardiac output and to limit the normal compensatory increase in systemic vascular resistance, both clinical features of CS.4 Thus, the presence of a systemic inflammatory syndrome in the setting of CS, with induction of both arginase and iNOS, may contribute to the high mortality observed.

Elevated plasma levels of ADMA have been reported in a large variety of patients with cardiovascular risk factors,34–37 CAD,38,39 preeclampsia,40 heart failure,41–43 and pulmonary hypertension,44 as well as in animal models of hypercholesterolemia,44 diabetes,45 and hypertension.46 Levels of ADMA are reported to correlate with the degree of experimental endothelial dysfunction,47 and ADMA was shown to exert numerous vascular effects, ranging from increased adhesion of endothelial cells to monocytes in vitro48 to increases in both endogenous oxidative stress and lesion formation in...
animal models after intravenous infusion.49 The endothelial dysfunction seen in heart failure also appears to be mediated, in part, by formation of ADMA in response to stimulation by circulating levels of endothelin-1.50 A protective effect of a number of therapeutic interventions on NO-dependent arterial reactivity has been reported to parallel reductions in systemic ADMA levels.35,51,52

Given that NOS inhibitors bind competitively with arginine, their biological effect will be increased in the setting of decreased tissue arginine levels. The observation that the ratios of arginine to ADMA and arginine to MMA are decreased in CS suggests that NOS activity is inhibited more effectively by these compounds in CS patients than in normal subjects. This decreased substrate-to-inhibitor ratio will tend to oppose the increased NO synthesis resulting from iNOS expression in response to inflammatory cytokine release. A conclusion as to which effect dominates cannot be drawn from the present analysis. However, the finding that systemic vascular resistance often is low in CS can be interpreted as resulting from overproduction of vasoactive NO. Overproduction of NO would imply a significant increase in NOS activity. Elimination of SDMA occurs primarily by renal excretion,53 and not surprisingly, its level is particularly elevated in CS patients with impaired renal function (Table 3). In contrast, ADMA and MMA undergo extensive metabolism in a process catalyzed by dimethylarginine dimethylaminohydrolase (DDAH) enzymes.54 The finding that ADMA and MMA are significantly elevated in CS patients with poor renal function may reflect the fact that the kidney is a major DDAH-containing organ. However, ADMA levels also were markedly elevated in CS subjects with normal renal function (Table 3), indicating that alternative mechanism(s) can contribute to the observed increased level of ADMA in CS. Overexpression of DDAH in animal models leads to a reduction in ADMA levels with beneficial effects on angiogenesis,55,56 systemic vascular resistance, and cardiac stroke volume.57 DDAH is sensitive to S-nitrosylation with consequent loss of activity, and this has been reported to occur after cytokine-induced expression of iNOS in cultured vascular endothelial cells.58 Thus, the generation of oxidative and nitrosative stress in the setting of systemic inflammatory states such as that seen in CS might have a profound impact on circulating levels of ADMA, both increasing its synthesis and decreasing its metabolism.

In the present studies, abnormal levels of NO-related arginine metabolites correlated with both mortality and hemodynamic abnormalities. Of particular interest, levels of ADMA correlated with 30-day mortality. This correlation was independent of subsequent treatment modality (placebo or MMA) or MMA dose, and it persisted in a multivariate model that took into account age, body mass index, estimated creatinine clearance, and systolic blood pressure, in addition to ADMA levels. Within the CS patient group, higher levels of ADMA correlated with increased mortality. Whether elevated ADMA levels are causally linked to the enhanced mortality risk cannot be ascertained with the present observational study. It appears more likely based on the many links between DDAH expression and activity levels and indexes of inflammation and oxidant stress, that CS patients with greater elevations of their ADMA levels may have had higher levels of systemic inflammation both pre-MI and post-MI, but before the onset of CS. This relationship may account for the observed correlation between high plasma ADMA levels and mortality. Regardless, our results suggest that elevated ADMA levels may serve as a useful prognostic marker in CS, predicting an increased risk of mortality. The possibility that elevated ADMA levels also may predict risk for CS among acute MI patients also warrants investigation.

Elevated levels of ADMA also correlated with increased pulmonary capillary wedge pressure and increased systolic and diastolic pulmonary pressures. The inverse correlation between ADMA and mean arterial pressure was statistically less robust (P=0.06) and is opposite of what would be expected if ADMA were an effective NOS inhibitor in this setting. It may be that high ADMA levels reflect sicker patients with enhanced catabolic state and/or greater induction of iNOS by the inflammatory cytokines. It is therefore interesting to note that ADMA levels also are elevated in the setting of septic shock, being highest in those who require hemodynamic support.59

One limitation of the present findings is that they result from multiple statistical comparisons in a relatively small cohort of patients with MI complicated by CS. It is uncertain whether this relationship is altered in settings other than CS resulting from left ventricular failure that persists despite establishment of infarct-related artery patency. Nonetheless, the present study represents one of the largest studies on CS subjects of its kind; it is the first to provide a comprehensive assessment of multiple metabolites involved in arginine and NO pathways. Although the study cohort included patients who were treated with different doses of the NOS inhibitor L-MMA or placebo, all arginine and NO metabolites were assessed in pretreatment samples. Treatment would thus have had no influence on the observed relationships between arginine or its metabolites and hemodynamic parameters because all analytes were measured before administration of either treatment. Although treatment could affect correlations with mortality, L-MMA treatment in SHOCK-217 and the Tilarginine Acetate Injection in a Randomized International Study in Unstable Patients with Cardiogenic Shock (TRIUMPH)60 did not show a statistically significant mortality effect, although there was a trend toward interaction with renal insufficiency (potential harm) in the latter study.61 However, pretreatment ADMA levels showed similar correlations with mortality in patients treated with either placebo or any dose of the exogenous NOS inhibitor L-MMA, and ADMA was independently associated with 30-day mortality in a multivariate model that included L-MMA administration. It should be noted that the present study was underpowered to investigate the effect of treatment on clinical outcome and that although these findings raise the possibility that ADMA may predict mortality and response to therapy in a larger trial or particular patient subset, this possibility awaits further investigation. Investigation of the ability of ADMA to identify patients who might benefit from therapy in the recently
completed larger TRIUMPH study is of interest for future study. It also remains to be determined whether successful clinical response to intensive management with medical therapies and revascularization is associated with an improvement in the derangements of these biomarker levels. Demonstrated normalization of NO-related arginine metabolites with successful treatment would further support the use of NO and arginine pathway metabolic profiling as either a therapeutic target or a marker for risk stratification. Finally, we note that concomitant therapies would affect hemodynamic parameters in the CS patient cohort. Inclusion criteria for sample collections from patients required patients to have significant clinical features of CS despite the use of cardiovascular support. As a result, hemodynamic parameters were measured in the setting of substantial circulatory assistance with vasopressors, inotropes, and intra-aortic balloon pumps and most often after percutaneous coronary intervention.

Conclusions

Systemic levels of a number of key NO-related arginine metabolites and protein products of oxidative stress are altered in the setting of CS. Our results show that some of these factors correlate with hemodynamic measures of disease severity and suggest that elevated ADMA levels independently predict mortality. These findings highlight the importance of NO in CS and add further evidence to the concept that identification and targeting of the specific neurohumoral events that contribute to the pathogenesis of pump failure may be important in the development of effective therapeutic strategies to improve clinical outcome.

Acknowledgment

Samples and access to clinical data from the SHOCK-2 trial were generously provided by Arginox Pharmaceuticals.

Sources of Funding

This work was supported by National Institutes of Health grants P01 HL076491, P01 HL77107, HL70621, and HL081064 and the Cleveland Clinic Foundation General Clinical Research Center (M01 RR018390). Dr Nicholls was supported by a Ralph Reader Overseas Research Fellowship from the National Heart Foundation of Australia. The MS reference laboratory directed by Dr Hazen received funding from Arginox Pharmaceuticals for quantification of ADMA, MMA, and arginine for the SHOCK-2 trial.

Disclosures

Dr Nicholls has received honoraria from Pfizer, AstraZeneca, Merck Schering-Plough, and Takeda and consulting fees from Pfizer, AstraZeneca, Roche, LipoScience, and Anthera. Dr Levinson owns shares in Merck and is a consultant to Prognostix. Dr Griffith owns shares in Arginox, holds patents for the use of NOS inhibitors to treat hypotension, and is an employee of the University of Wisconsin, which has an ownership interest in Arginox. Dr Hathaway is a former employee of Arginox. Dr Dzavik has received research support from Arginox and speaking honoraria from Datascop and Arginox. Dr Panza has received research support from Arginox and Takeda and is a member of the speakers’ bureau of Pfizer and Glaxo Smith Kline. Dr Nissen has received research support from Takeda, Sanofi-Aventis, Eli Lilly, Pfizer, Sankyo, Atherogenics, Lipid Sciences, and AstraZeneca and provides consulting for a number of pharmaceutical companies but requires them to donate all honoraria or consulting fees directly to charity so that he receives neither income nor tax deductions. Dr Hochman has conducted clinical trials sponsored by Arginox. The other authors report no conflicts.

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**CLINICAL PERSPECTIVE**

This study investigated whether abnormalities of arginine and nitric oxide metabolism and protein oxidation correlate with hemodynamic dysfunction and 30-day mortality rates in subjects with cardiogenic shock (CS). CS patients had higher levels of ornithine, citrulline, and the methylated arginine derivatives asymmetric dimethylarginine, symmetric dimethylarginine, and monomethylarginine; lower levels of arginine; and markedly higher levels of multiple specific molecular markers of oxidant stress than age- and gender-matched subjects with coronary artery disease and normal left ventricular function. Among all clinical and laboratory parameters monitored, asymmetric dimethylarginine remained the only independent predictor of mortality on multivariate analysis in CS. Symmetric dimethylarginine and asymmetric dimethylarginine levels demonstrated significant associations with multiple hemodynamic parameters. These findings are the first to show diminished arginine bioavailability and enhanced oxidant stress in CS and to identify elevated levels of endogenous nitric oxide synthase inhibitors as being of prognostic importance in CS.
Metabolic Profiling of Arginine and Nitric Oxide Pathways Predicts Hemodynamic Abnormalities and Mortality in Patients With Cardiogenic Shock After Acute Myocardial Infarction

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* Circulation.* 2007;116:2315-2324; originally published online October 29, 2007; doi: 10.1161/CIRCULATIONAHA.107.693986

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/116/20/2315

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