Asymmetrical Dimethylarginine in Idiopathic Pulmonary Arterial Hypertension

Jan T. Kielstein, Stefanie M. Bode-Böger, Gerrit Hesse, Jens Martens-Lobenhoffer, Attila Takacs, Danilo Fliser, Marius M. Hoeper

Objective—We explored the potential role of the endogenous NO synthase inhibitor asymmetrical dimethylarginine (ADMA) in patients with idiopathic pulmonary arterial hypertension (IPAH).

Method and Results—We correlated plasma ADMA levels and cardiovascular indices from right heart catheterization in 57 patients with IPAH. Predictors of survival in patients with IPAH were studied. Furthermore, the effect of systemic ADMA infusion on pulmonary ventricular resistance and stroke volume was investigated in healthy volunteers using right heart catheterization. Mean plasma ADMA concentrations were significantly higher in patients with IPAH than in control subjects (0.53±0.15 versus 0.36±0.05 μmol/L; P<0.001). ADMA plasma concentrations correlated significantly with indices of right ventricular function, such as mixed-venous oxygen saturation (r=-0.49; P<0.0001), right atrial pressure (r=0.39; P<0.003), cardiac index (r=-0.35; P<0.008), as well as survival (r=-0.47; P<0.0001). Multiple regression analysis revealed that right atrial pressure (r=0.31; P<0.026) and ADMA (r=0.29; P<0.039) were independent predictors of mortality. Moreover, patients with supra-median plasma ADMA levels had significantly (P<0.021) worse survival than patients with infra-median ADMA values. ADMA infusion in healthy volunteers increased pulmonary vascular resistance (68.9±7.6 versus 95.6±6.3 dyne·s·cm⁻²; P<0.05) and decreased stroke volume (101.1±6.7 mL versus 95.6±6.3 mL; P<0.05).

Conclusion—Increased ADMA plasma levels are associated with unfavorable pulmonary hemodynamics and worse outcome in patients with IPAH. (Arterioscler Thromb Vasc Biol. 2005;25:1414-1418.)

Key Words: idiopathic pulmonary arterial hypertension ■ ADMA ■ nitric oxide

Idiopathic pulmonary arterial hypertension (IPAH) is a disease of unknown etiology that is characterized by progressive obliteration of small and medium-size pulmonary arteries, elevation in pulmonary arterial pressure, and increase in pulmonary vascular resistance, eventually leading to right heart failure and death. Without appropriate medical treatment, mean survival of patients experiencing IPAH is <3 years. In the current understanding of the pathogenesis of IPAH, it is thought that a combination of genetically determined predisposition coupled with ill-defined exogenous factors may contribute to damage of the pulmonary vessels, resulting in endothelial dysfunction with proliferation of vascular endothelial and smooth muscle cells. Endothelial dysfunction attributable to reduced bioavailability of endogenous vasodilator substances such as NO is thought to play an important role in the development of pulmonary hypertension. NO is synthesized in the endothelium from the amino acid L-arginine by the action of NO synthase (NOS). Endogenous guanidino-substituted analogues of L-arginine that can selectively inhibit NOS have been implicated in the pathogenesis of various cardiovascular diseases. The most potent of these endogenous NOS inhibitors is asymmetrical dimethylarginine (ADMA). Elevated ADMA levels and low L-arginine/ADMA ratios not only correlate with the severity of atherosclerotic disease but also predict cardiovascular outcome and overall mortality. We demonstrated recently that pathophysiologically relevant ADMA blood concentrations inhibited NO production and reduced cardiac output and renal perfusion in healthy subjects. Experimental data from a rat model of chronic hypoxia-induced pulmonary hypertension revealed that increased pulmonary concentrations of ADMA contributes to pulmonary hypertension. On the basis of these observations, it seems possible that increased plasma ADMA concentrations may also contribute to endothelial dysfunction in patients with pulmonary vascular disease. The present study was undertaken to evaluate a potential role of ADMA in IPAH.

Subjects and Methods

Participants and Protocols
The study protocol was approved by the local Ethics committee, and all participants gave informed consent. We recruited 57 whites (19...
males; 49±14 years) with severe IPAH in functional class III and IV according to the diagnostic criteria defined by a World Health Organizinat expert panel.17 For comparison, 22 subjects (9 males; 48±14 years) without relevant medical problems matched with respect to renal function, mean arterial pressure, and cholesterol and triglyceride levels were examined. All patients underwent a right heart catheter examination as part of the initial diagnostic workup at the time of the first diagnosis of pulmonary hypertension. Details of the catheter procedure have been described previously.18 None of the patients were under treatment with nitrates, NO donors, prostanoids, endothelin receptor antagonists, or sildenafil at the time of diagnostic cardiac catheterization. No other relevant medical problems were present at that time. Blood samples for measurement of plasma ADMA concentrations were obtained during heart catheterization. Blood samples were immediately cooled on ice, centrifuged at 1500g and 4°C for 10 minutes, and the supernatants were stored in 1 mL aliquots at −80°C until further use.

After completion of the diagnostic cardiac catheterization, all patients were further followed to assess survival. Patients were recruited for this study between February 2000 and September 2001 and followed until September 2003 or until death. All patients were treated with oral anticoagulants unless contraindicated. One patient was considered a “responder” after acute vasodilator challenge and received calcium channel blockers. The remaining patients were nonresponders in functional class New York Heart Association III or IV and were treated with inhaled, oral, or intravenous prostanoids, or endothelin receptor antagonists.

ADMA Infusion
The study protocol was separately approved by the ethics committee of the Hannover Medical School. Written informed consent was given by all participants, who were healthy nonsmoking male volunteers. A right heart catheter was placed under local anesthesia, and hemodynamic measurements were described as previously.16 After an appropriate equilibration period to minimize stress after catheter placement, hemodynamics were assessed continuously during placebo infusion period (50 minutes), followed by intravenous infusion of 0.1 mg/kg per minute ADMA for 40 minutes. Hemodynamics were monitored continuously for another 120 minutes after discontinuation of ADMA infusion.

Measurements and Calculations
Plasma concentrations of ADMA were measured applying a recently developed liquid chromatography-mass spectrometry method described previously.19 Briefly, after addition of the internal standard solution (13C6-arginine and homoarginine), 250 μL plasma was deproteinized by the addition of 0.5 mL acetonitril, the supernatant was evaporated to dryness, and the residue was redissolved in formic buffer. Samples were automatically derivatized with 2-mercaptoethanol reagent and were analytically separated on a Merck Superspher RP-18 250×4 mm high-performance liquid chromatography column, applying a formic buffer/methanol gradient. All analytes were separated sufficiently and detected selectively by a ThermoFinnigan LCQ mass spectrometer equipped with an electrospray ionization ion source. The coefficient of variation is 7.5%. The method was validated according to the guidelines for biochemical assays.20 Plasma total homocysteine concentrations were measured with a fluorescence-polarization immunoassay. All other measurements were done with routine laboratory tests using certified assay methods.

Statistical Analysis
We used SPSS for statistical analysis (SPSS 11.51 for Windows). The normality of data distribution was confirmed with the Shapiro–Wilk test. Comparison between groups was done using a 2-tailed t test for comparison of random data. Pearson’s correlation analyses were performed between plasma ADMA concentrations on the one hand, and age, right heart catheter indices, and survival (categorized as dead/alive) on the other. In addition, ADMA, age, and right heart catheter indices were applied in a multiple regression analysis to reveal independent determinants of survival. Moreover, we analyzed survival in patients with IPAH using a log-rank analysis and created Kaplan–Meier survival curves. For this purpose, the group of patients was divided into those with a supramedian ADMA level and those with plasma ADMA concentrations below the median value. Data are presented as mean±SD.

A paired t test was used to compare the intradividual preinfusion (minute 50) and postinfusion (minute 90) cardiovascular parameters obtained during the right heart catheter experiments. The significance level was set at P<0.05.

Results
Mean plasma ADMA concentrations were significantly higher in patients with IPAH than in control subjects (0.53±0.15 versus 0.36±0.05 μmol/L; P<0.001 μmol/L; Figure 1).

Hemodynamic parameters obtained during right heart catheter examination in patients with IPAH are shown in Table 1. Results of the correlation analyses between plasma ADMA concentration and age, right heart catheter indices, and survival (categorized as dead/alive) are summarized in Table 2. Plasma ADMA levels significantly correlated with mixed-venous oxygen saturation (Figure 2A), right atrial pressures (Figure 2B), pulmonary vascular resistance, stroke volume, cardiac index, and survival (Table 2). There was no significant difference between IPAH patients and controls with regard to cholesterol and triglyceride and blood glucose levels; Table I, available online at http://atvb.ahajournals.org

Although the mean homocysteine plasma level was within the normal range in both groups, IPAH patients tended to have higher mean serum homocysteine levels (13.2±4.0 versus 10.5±3.4 μmol/L; P<0.05).

| TABLE 1. Data From Right Heart Catheter Examination in 57 Patients With IPAH |
|------------------------|-----------------|------------------------|-----------------|
|                        | Mean arterial blood pressure, mm Hg | Right atrial pressure, mm Hg | Mean pulmonary arterial pressure, mm Hg |
|                        | 94±14 | 9.0±6.2 | 54±14 |
| Pulmonary vascular resistance, dyne·s·cm⁻³ | 1101±549 | Stroke volume, mL | 49±18 |
| Cardiac index, L·min⁻¹·m⁻² | 2.0±0.7 | Systemic vascular resistance, dyne·s·cm⁻³ | 1945±726 |
| Systemic oxygen saturation, % | 64±11 | Mixed-venous oxygen saturation, % | 60±9 |

All measurements were made without supplemental oxygen.
TABLE 2. Results of Correlation Analyses With Plasma ADMA Concentration on the One Hand, and Age, Right Heart Catheter Indices, and Survival (categorized as dead/alive) on the Other

<table>
<thead>
<tr>
<th>Plasma ADMA concentration</th>
<th>Mixed-venous oxygen saturation</th>
<th>Survival</th>
<th>Right atrial pressure</th>
<th>Stroke volume</th>
<th>Cardiac index</th>
<th>Mean arterial blood pressure</th>
<th>Pulmonary vascular resistance</th>
<th>Systemic vascular resistance</th>
<th>Age</th>
<th>Pao2</th>
<th>Mean pulmonary arterial pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r = -0.49</td>
<td></td>
<td>r = -0.47</td>
<td>r = 0.39</td>
<td>r = -0.35</td>
<td>r = 0.35</td>
<td>r = 0.32</td>
<td>r = 0.24</td>
<td>r = -0.35</td>
<td>r = 0.10</td>
<td>r = -0.07</td>
</tr>
</tbody>
</table>

The median follow-up period in patients with IPAH after completion of the diagnostic right heart catheterization was 26 (1 to 44) months. During this follow-up period, 18 patients died. Clinical data and results of the right heart catheter examination from survivors and nonsurvivors are presented in Table 3. On stepwise multiple regression analysis, only right atrial pressure (r = 0.31; P = 0.026) and ADMA (r = 0.29; P = 0.039) were independent predictors of mortality after inclusion of age, mean pulmonary arterial pressure, cardiac index, stroke volume, mean arterial blood pressure, pulmonary vascular resistance, and mixed-venous oxygen saturation. Figure 3 presents Kaplan–Meier survival curves for patients with IPAH. In patients with supramedian values of plasma ADMA concentrations (ie, >0.51 μmol/L), survival was significantly worse (P = 0.021) than in patients with plasma ADMA levels below the median value.

ADMA infusion in healthy volunteers increased pulmonary vascular resistance (68.9 ± 7.6 versus 95.6 ± 6.3 dyne · s · cm⁻²; P < 0.05) and decreased stroke volume (101.1 ± 6.7 versus 95.6 ± 6.3 mL; P < 0.05; Figure IA and IB, available online at http://atvb.ahajournals.org).

Discussion

The major novel findings of the present study are: (1) plasma ADMA levels in patients with IPAH were significantly higher than in matched healthy controls; (2) in patients with IPAH, plasma ADMA concentrations correlate significantly with predictors of survival such as right atrial pressure and mixed-venous oxygen saturation; and (3) ADMA itself is an independent predictor of survival in patients with IPAH. These observations lead to several questions. Is ADMA a pathophysiologically relevant mediator or simply an innocent

Figure 2. Scatter plots of the relationship between plasma ADMA concentration. Mixed-venous oxygen saturation (A; r = -0.49; P < 0.0001) and right atrial pressure (B; r = -0.39; P < 0.003), in 57 patients with IPAH.

Figure 3. Kaplan–Meier survival curves in patients with IPAH and supramedian values of plasma ADMA concentrations (n = 28) and inframedian plasma ADMA values (n = 29). In patients with supramedian values of plasma ADMA concentrations (ie, >0.51 μmol/L), survival was significantly worse (P < 0.021).
bystander in this disease, and do these findings have potential therapeutic implications?

Is ADMA a relevant factor in the pathogenesis of IPAH? Firm conclusions from our data would be premature in this respect. However, the results of the infusion study suggest that acute administration of ADMA increases pulmonary vascular resistance and decreases stroke volume. A decrease in cardiac output by ADMA infusion had been shown previously.14 This finding is in line with data on the multifaceted involvement of NO in cardiac (patho)physiology; small increments in NO concentration have a positive inotropic effect, and conversely, inhibition of NOS may result in a negative inotropic effect.21 Above that, a very recent study showed that long-term (4-week) application ADMA can indeed cause significant microvascular lesions in mice.22

ADMA is released from proteins that have been post-translationally methylated and subsequently hydrolyzed. To some extent, ADMA is renally excreted, but the major metabolic pathway is degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which hydrolyzes ADMA to dimethylamine and L-citrulline.23 A recent study in transgenic mice that overexpress DDAH provided compelling evidence that the metabolism of ADMA plays an important role in regulation of NOS activity.24 A relevant role of ADMA in the pathogenesis of IPAH could derive from reduced activity of DDAH with increasing severity of the disease, eventually as a result of increased oxidant stress as well as hypoxia.9 This assumption is corroborated by findings from several studies. Hypoxia in severe hemorrhagic shock has been shown to lead to an increase in ADMA in a pig model.25 More specifically, in a hypoxia-induced pulmonary hypertension rat model, chronic hypoxia leads to an increase in ADMA caused by a reduced pulmonary expression and activity of DDAH.15 The patients in the present study had a mean mixed venous oxygen saturation of 60%, reflecting some degree of tissue hypoxia, which could have contributed to increased ADMA levels. Furthermore, in a pig model of pulmonary hypertension of the newborn, it was shown that the underlying mechanism for ADMA elevations is suppression of DDAH expression and activity.26

Another possible mechanism of reduced DDAH activity might be related to plasma homocysteine concentrations. Recently, Stuehlinger et al reported that homocysteine in concentrations >30 μmol/L inhibits the activity of DDAH, leading to an accumulation of ADMA in vitro.27 Consistent with a previous study,28 plasma homocysteine levels in IPAH were within the normal range (ie, between 5 and 15 μmol/L) but significantly elevated compared with controls. It is possible that concentrations as low as 15 μmol/L might influence DDAH activity and subsequently increase ADMA concentrations because already physiological increments in plasma homocysteine have been shown to induce vascular endothelial dysfunction in normal human subjects.29

Finally, an alternative explanation for increased ADMA blood concentrations in IPAH patients with higher mortality would be that they simply reflect severe injury of the pulmonary vascular bed because endothelial cells can produce large amounts of ADMA.30

Our findings that ADMA is an independent predictor of mortality in patients with IPAH is reminiscent of the results of 2 landmark studies showing that increased ADMA predicts cardiovascular and overall mortality in patients with end-stage renal disease as well as in patients with coronary heart disease.12,13 Furthermore, it fits into a rapidly growing number of clinical studies documenting a strong correlation between ADMA and cardiovascular morbidity and mortality in different populations.10,31–33 Several studies in different populations have yielded ample evidence that even small increases in plasma ADMA levels are associated with deterioration of endothelial function and a significant increase in the rate of cardiovascular events.12,13,36 Accordingly, we found a significant correlation between ADMA and right atrial pressure as well as cardiac index, both of which are important prognostic parameters in IPAH. All in all, the pathophysiological role of ADMA in IPAH still remains to be unfolded in detail, even more so because the relationship between the ADMA/NO system is incompletely understood and probably much more complex than currently known.

Is the ADMA/NO system a potential therapeutic target in IPAH? Because L-arginine is a potent NO donor, it is tempting to speculate that raising the plasma L-arginine concentration might attenuate the detrimental effects of ADMA in patients with IPAH. Two studies have addressed L-arginine supplementation in patients with IPAH with conflicting results. Short-term infusion (ie, 15 minutes) of high-dose L-arginine had no hemodynamic effect in 5 patients with IPAH.37 In contrast, in a placebo-controlled study in 19 patients with IPAH, 1-week oral L-arginine supplementation resulted in improved exercise capacity and hemodynamics.38 A multicenter trial to establish the potential therapeutic role of L-arginine supplementation in IPAH has been concluded recently but, apparently, results have been negative (these data have not yet been published). Thus, there is not enough evidence to recommend L-arginine supplements for patients with IPAH.

In conclusion, the significant correlation of plasma ADMA concentrations with important indices of pulmonary hemodynamics, together with the predictive power of ADMA for survival of patients with IPAH as well as the short-term effect of ADMA infusion on the pulmonary circulation in healthy men, suggests that ADMA may be a relevant factor in the pathogenesis of IPAH.

Acknowledgments

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References


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Figure I A

[Graph showing changes in PVR (dynes.s.cm^-5) over time (min) with an ADMA infusion.]

- PVR (dynes.s.cm^-5)
- Time (min)
Figure I B

The graph illustrates the change in stroke volume (mL) over time (min) during ADMA infusion. The data points are represented with error bars indicating variability. A significant change is observed at approximately the 60-minute mark, marked by an asterisk (*) on the graph.
Figure I. Effect of 0.10 mg ADMA/kg/min on pulmonary vascular resistance (Figure I A) and stroke volume (Figure I B) in 7 healthy volunteers. * = p<0.05 - comparison between pre-infusion (baseline) and post-infusion data. Data are presented as mean ±SEM. Please see www.ahajournals.org
Table I. Cardiovascular risk factors and characteristics of patients with IPAH and controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>IPAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>57</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 ± 14</td>
<td>49 ± 14</td>
</tr>
<tr>
<td>Male/female</td>
<td>9/13</td>
<td>19/38</td>
</tr>
<tr>
<td>cholesterol (mg/dL)</td>
<td>186 ± 24</td>
<td>163 ± 36</td>
</tr>
<tr>
<td>triglyceride (mg/dL)</td>
<td>110 ± 61</td>
<td>122 ± 47</td>
</tr>
<tr>
<td>blood glucose (mmol/L)</td>
<td>4.6 ± 0.8</td>
<td>4.7 ± 0.6</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>10.5 ± 3.4</td>
<td>13.2 ± 4.0*</td>
</tr>
</tbody>
</table>

* - p<0.05 – comparison between controls and IPAH patients