Asymmetric dimethyl-arginine (ADMA) response to inflammation in acute infections

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Abstract

Background and methods. The endogenous inhibitor of nitric oxide synthase (NOs) asymmetrical dimethyl-arginine (ADMA) has been implicated as a possible modulator of inducible NOs during acute inflammation. We examined the evolution in the plasma concentration of ADMA measured at the clinical outset of acute inflammation and after its resolution in a series of 17 patients with acute bacterial infections.

Results. During the acute phase of inflammation/infection, patients displayed very high levels of C-reactive protein (CRP), interleukin-6 (IL-6), procalcitonin and nitrotyrosine. Simultaneous plasma ADMA concentration was similar to that in healthy subjects while symmetric dimethyl-arginine (SDMA) levels were substantially increased and directly related with creatinine. When infection resolved, ADMA rose from 0.62 \pm 0.23 to 0.80 \pm 0.18 \text{ mol/l} ( +29\%, P = 0.01) while SDMA remained unmodified. ADMA changes were independent on concomitant risk factor changes and inversely related with baseline systolic and diastolic pressure. Changes in the ADMA/SDMA ratio were compatible with the hypothesis that inflammatory cytokines activate ADMA degradation.

Conclusions. Resolution of acute inflammation is characterized by an increase in the plasma concentration of ADMA. The results imply that ADMA suppression may actually serve to stimulate NO synthesis or that in this situation plasma ADMA levels may not reflect the inhibitory potential of this methylarginine at the cellular level.

Keywords: asymmetrical dimethyl-arginine; C-reactive protein; inflammation; interleukin-6; procalcitonin

Introduction

Asymmetrical dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase (NOs) which has now fully emerged as a risk marker in a variety of conditions including end-stage renal disease [1], liver insufficiency [2], heart failure [3], diabetes [4], pre-eclampsia [5], and atherosclerotic complications [6]. Inflammation, as measured by C-reactive protein (CRP), is another strong risk marker which predicts death and cardiovascular complications in disparate diseases including inflammatory diseases [7], sepsis [8], pulmonary diseases [9] and coronary heart diseases [10]. The interplay between ADMA and inflammation is a relevant issue because both factors, ADMA and CRP, have been implicated in endothelial dysfunction in humans [11,12]. Observations in healthy individuals show that a very brief exposure to an endotoxin or to certain cytokines impairs endothelium-dependent relaxation for many days [13]. This phenomenon has been attributed to the fact that in the presence of superoxide anion, a prominent product of the inflammatory response, NOs forms peroxynitrite. Peroxynitrite may cause extensive organ damage because it elicits nitrosation of proteins and enzymes or because it irreversibly inhibits mitochondrial respiration (nitrosative stress) [14]. Reducing NO generation in this situation may, in theory, limit inflammation-related ‘nitrosative stress’. In this perspective, the endogenous inhibitor of NO synthase ADMA may be seen as a factor physiologically aimed at countering the adverse effects of inflammation on the endothelium during inflammatory processes. Such a possibility would be supported by observations showing that the infusion of bacterial lipopolysaccharide (LPS) to young healthy individuals, while not modifying the plasma concentration of ADMA, induces a decrease in the L-Arginine/ADMA ratio, i.e. lowers the potential for endogenous NO generation [15]. On the other hand,
experiments in the rat demonstrated that plasma ADMA is actually reduced during endotoxinemia by *Escherichia coli* (*E. coli*) LPS [16] suggesting that ADMA modulation may serve to blunt rather than to increase NOs activity during inflammation driven by infectious processes. We thought that direct study of the evolution of inflammation secondary to acute infections may represent a natural human model for exploring the effect of inflammation on ADMA. In this longitudinal study we have therefore examined the evolution in the plasma concentration of ADMA measured at the clinical outset of acute inflammation and as the acute process subsided in a series of patients with bacterial infections.

**Methods**

The study was approved by the Ethics Committee of our institution and informed consent was obtained by each participant. Enrolled in this study were 17 consecutive patients (age 55 ± 19 years; 6 women and 11 men) admitted to the medical ward of our tertiary care hospital because of high fever (>39°C), chills and markedly raised serum CRP. Demographic data, renal function and other pertinent biochemical data of patients and controls are reported in (Table 1). All of these patients had underlying chronic diseases including coronary heart diseases, chronic heart failure, chronic broncho-pulmonary diseases, diabetes mellitus and lupus erythematosus, and all exhibited a variable degree of renal insufficiency but no patient was on chronic dialysis. In all cases, the cause of inflammation was represented by bacterial infections. The diagnosis of bacterial infection was made on the basis of positive blood cultures and/or on the presence of high levels of plasma procalcitonin (an indicator of bacterial infection [17]) associated with a marked fall of this biomarker after empiric antibiotic therapy. Eight patients had broncho-pulmonary or urinary infections, two had septic arthritis, two limb abscesses, one infected central catheter, one brucellosis, one infection coincident with lupus erythematosus reactivation, and two infections superimposed to influenza. In no patient was there evidence of severe multi-organ failure, i.e. evidence of acute liver damage or severe respiratory insufficiency or marked hypotension.

Blood sampling was done during the first 3 h after admission into the clinical ward, before commencing any specific treatment. Thereafter, patients were managed by attending physicians who monitored the severity of inflammation clinically and by measuring serum CRP and procalcitonin and instituting appropriate antibiotic treatment. Steroids were used occasionally in small doses on the first or second day in patients with infection while prednisone was used in high doses in the patient with systemic lupus reactivation. The second sample was taken after full resolution of fever, i.e. at least 3 days after defervescence, before discharge.

**Laboratory methods**

Blood was drawn and put into tubes containing EDTA, and plasma supernatants were stored at 70–80°C until batch analyses. All analyses were done blinded to clinical information and sequential timing of sampling. High sensitivity CRP was measured by a commercially available kit (Dade Behring, Marburg, Germany, intra-assay coefficient of variation (CV): 3.5%; inter-assay CV: 3.4%; 95th percentile of the normal range: 2.9 ng/ml.). Serum levels of IL-6 and TNF-α were measured by an ELISA with the use of Quantikine High Sensitivity kit (IL-6, intra-assay CV: 2.6%; inter-assay CV: 4.5%; upper limit of the normal range: 12.5 pg/ml; TNF-α, intra-assay CV: 4.7%; inter-assay CV: 5.8%; upper limit of the normal range: 15.6 pg/ml) (R&D Systems Inc, MN, USA). Procalcitonin, an established marker of bacterial infection [17], was measured by a commercially available system (Kryptor PCT, Brahms, Hennigsdorf, Germany; intra-assay CV: 3%; inter-assay CV: 5%; 95th percentile of the normal range: 0.06 ng/ml). Nitrotyrosine was determined in serum by using the ELISA by HyCult Biotechnology (Uden, The Netherlands) (normal values <2 nmol/l). Cholesterol, triglycerides, albumin, haemoglobin and creatinine were measured in the central hospital laboratory that applies internationally recommended guidelines for quality control.

**Table 1. Baseline characteristics of patients**

<table>
<thead>
<tr>
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<th>Patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>55 ± 19 SD</td>
<td>55 ± 8 SD</td>
</tr>
<tr>
<td>Sex</td>
<td>11 M and 6 F</td>
<td>22 M and 12 F</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6 ± 3.9 SD</td>
<td>27.1 ± 3.0 SD</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>8 (47%)</td>
<td>16 (47%)</td>
</tr>
<tr>
<td>Diabetics (%)</td>
<td>6 (35%)</td>
<td>2 (5.9%)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>132 ± 38 SD</td>
<td>222 ± 47 SD</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>154 (90–209)</td>
<td>126 (66–147)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.09 ± 0.56 SD</td>
<td>NA</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.4 ± 1.9 SD</td>
<td>NA</td>
</tr>
<tr>
<td>Leucocytes n (×10⁹)</td>
<td>14 ± 7 SD</td>
<td>NA</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>2.9 (1.1–6.8)</td>
<td>1.0 (0.8–1.2)</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>132 ± 26 SD</td>
<td>133 ± 16 SD</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>77 ± 12 SD</td>
<td>83 ± 8 SD</td>
</tr>
<tr>
<td>Heart rate (b/min)</td>
<td>96 ± 20 SD</td>
<td>72 ± 9 SD</td>
</tr>
<tr>
<td>GFR, MDRD (ml/min/1.73 m²)</td>
<td>27 (8–45)</td>
<td>77 (65–92)</td>
</tr>
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SD: standard deviation; GFR: the glomerular filtration rate was estimated by modification of diet in renal disease (MDRD) study group formula [29].

**L-Arginine, ADMA, and SDMA (symmetrical dimethylarginine)**

L-Arginine, ADMA, and SDMA were determined by high sensitive liquid chromatography-tandem mass spectrometry with between-run CVs of 4.7% for L-arginine, 4.1% for ADMA, and 3.9% for SDMA as previously published and validated in detail [18]. The control group for plasma ADMA was formed of 34 matched (2:1) healthy controls from a multicenter population-based survey performed by us [19]. To this end we matched two healthy controls to each patient for age, gender, BMI and smoking status.

The ADMA/SDMA ratio was calculated to have a rough estimate of the DDAH activity [20], a low ratio being suggestive of high activity and vice versa.
Statistical analysis

Continuous variables are expressed as mean ± SD (normally distributed data) or as median and inter-quartile range (normally non-distributed data). Differences between the samples taken during acute inflammation/infection and after resolution of this process were analysed by paired \( t \)-test or Wilcoxon-matched pair’s test, as appropriate, and the corresponding point estimates were expressed as mean ± SE. The association between paired (unadjusted) variables was analysed by Pearson product moment correlation coefficient (\( r \)). A \( P \)-value of <0.05 was defined as the level of statistical significance. Data adjustment of changes in plasma ADMA for the corresponding baseline values as well as for creatinine, smoking, cholesterol and serum albumin was done by analysis of covariance and data were expressed as partial \( r \) and \( P \)-value. All calculations were done using a standard statistical package (SPSS for Windows Version 9.0.1, 11 Mar-1999, Chicago, IL, USA).

Results

Inflammatory markers, ADMA, and SDMA during the acute phase of inflammation/infection

During the acute phase of inflammation/infection, patients displayed very high levels of CRP which on average was over 100 mg/dl, i.e. a level two orders of magnitude higher than the average value in healthy subjects (Figure 1). The severity of inflammation was also documented by the parallel, substantial rise in IL-6 which attained a median value of 77 pg/ml. Procalcitonin, an indicator of bacterial infection and a marker of inflammation, was above the 95th percentile of the normal range in all patients. Nitrotyrosine was substantially elevated indicating ongoing nitrosative stress. Plasma ADMA concentration (0.62 ± 0.23 μmol/l) was similar to the mean plasma concentration of (0.65 ± 0.13 μmol/l).

Fig. 1. Scatter plots of CRP, IL-6, procalcitonin, nitrotyrosine, TNF-\( \alpha \), ADMA, SDMA and L-arginine during the acute phase of inflammation/infection. Because of the positively skewed distribution of CRP, IL-6 and procalcitonin these data are reported in log scale. The broken line coincides with the upper limit of the normal range for CRP, IL-6, procalcitonin, nitrotyrosine, TNF-\( \alpha \), ADMA and SDMA. The grey area identifies the normal range for L-arginine.
34 well-matched healthy controls from the general population.

Serum creatinine was raised in the majority of cases, the median value being 2.9 mg/dl (1.1–6.8 mg/dl). SDMA levels were substantially increased (average: 2.49 ± 1.94 μmol/l) and directly related to serum creatinine (r = 0.91, P < 0.01), while ADMA was unrelated to creatinine (P = 0.25). Average l-Arginine (115.6 ± 40.1 μmol/l) was in the normal range (Figure 1).

Inflammatory markers, ADMA, and SDMA after full resolution of fever

As expected, inflammatory markers fell considerably after the resolution of fever (CRP: −66%; procalcitonin: −84%; IL-6: −88%; TNF-α: −44%) (Figure 2). In parallel with inflammatory biomarkers decline, nitrotyrosine levels showed a marked (−60%) decrease pointing to amelioration in the oxidative stress status. Renal function improved as indicated by a 38% fall in serum creatinine but on average remained above 1.3 mg/dl in the majority of cases. Coincident with these changes, ADMA rose to 0.80 ± 0.18 μmol/l (＋29%, P = 0.01) as SDMA underwent a small (−0.3 μmol/l), non-significant decline again maintaining a strong, direct link with serum creatinine (r = 0.89, P = 0.001). ADMA changes adjusted for serum creatinine (＋29%) or for smoking, cholesterol and albumin (＋32%) did not materially differ from the crude estimate (＋29%, see above). The ADMA/SDMA ratio during the acute phase of inflammation/infection was 0.43 ± 0.32 and increased to 0.65 ± 0.47 after the resolution of fever suggesting that DDAH was much activated at the peak of fever and that this activation reversed when the infectious process resolved. Interestingly, ADMA changes were significantly associated with baseline systolic and diastolic pressure in an inverse fashion (Figure 3). l-Arginine rose to 135.5 ± 39.6 μmol/l (P = 0.03).

Discussion

This study shows that resolution of acute inflammation secondary to infectious processes is associated with a clear-cut rise in plasma ADMA levels coincident with a marked decline in biomarkers of inflammation and nitrosative stress, while SDMA levels remain unchanged throughout. The data imply that during infectious/inflammatory processes ADMA suppression may either serve to amplify NO synthesis or that in this condition extra-cellular (plasma) ADMA may not

Fig. 2. CRP, IL-6, procalcitonin, TNF-α, nitrotyrosine, ADMA, SDMA, l-arginine and creatinine during the acute phase of inflammation/infection and after its resolution. Data are expressed as mean ± SE.

Fig. 3. Relationship between baseline systolic and diastolic pressure with changes in plasma ADMA occurring between the acute phase of inflammation/infection and after its resolution. To avoid the statistical phenomenon of the ‘regression to the mean’, changes in plasma ADMA were adjusted for the corresponding baseline values. Data are expressed as partial r and P value.
reflect the inhibitory potential of this methylarginine at the intracellular level.

It was hypothesized that ADMA is fundamental for limiting cytokine-stimulated NO synthesis by inducible NOs but there is still no proof in favour of this intriguing hypothesis. Short term, intravenous administration of *E. coli* endotoxin in humans did not increase ADMA in healthy volunteers [15] while systemic plasma levels of ADMA actually decreased during continuous infusion of LPS in a rat model of endotoxemia [16]. To date there are no longitudinal, extended observations on plasma ADMA in patients with active inflammation sustained by infection. The question is relevant because in this specific situation it is still unknown whether the defensive properties (e.g. bacterial killing) of NO outweigh the risk posed by excessive nitro-oxidative stress and whether ADMA modulates this process.

Cytokines like IL-1 [21], IL-6 [22] and procalcitonin [23] all amplify the expression of inducible NOs as well as NO production. In the present study, in patients with acute, febrile, inflammation secondary to infection, we found that coincident with peak levels of IL-6, procalcitonin and CRP, plasma ADMA concentration was substantially lower than during the recovery phase of inflammation. In contrast to ADMA, SDMA remained unmodified and strongly related to serum creatinine which is in keeping with a recent meta-analysis by Kielstein et al. [24].

It is important to emphasize that our study setting is much different from severe septicemia associated with multi-organ failure, a disease with a very high fatality rate where ADMA is markedly raised mainly due to hepatic failure [25]. The relative benignity of infection in our series was proven by the fact that infection resolved in all cases and no short-term death was registered. Pro-inflammatory cytokines in vitro have a dual effect on the ADMA-inducible NOs system [26]. Indeed IL-1 on one hand stimulates dose-dependently inducible NO synthase and on the other hand determines a parallel stimulation of DDAH which would tend to decreased ADMA levels. In an experiment in the rat with LPS that mimicked the clinical situation in humans, ADMA was significantly lower in rats with endotoxinemia than in control animals, a phenomenon accompanied by increased ADMA turnover [16]. Intriguingly, in this study reduced ADMA levels could not be attributed to increased renal elimination because renal function was reduced during LPS infusion. Similarly, we found that lower ADMA levels during the acute phase of inflammation/infection coincided with higher creatinine levels documenting an acute impairment in renal function. Even though the kidney is not the major organ disposing ADMA, reduced renal function would tend to raise rather than to decrease ADMA. Although it was reported that TNF-α in the endothelial cell in vitro blunts DDAH activity without modifying the expression of this enzyme [27], cytokines stimulate both DDAH activity and expression in cultured smooth muscle cells (α-cellular species expressing iNOS) and determine a decrease in the ADMA content of culture media [26]. Thus it seems likely that the very high levels of cytokines during inflammation in our patients suppressed ADMA levels by enhancing DDAH activity and that this suppression reversed as the inflammatory/infectious process subsided. This interpretation would be in line with our observation that the ADMA/SDMA ratio, a rough indicator of DDAH activity [20], rose markedly when the inflammatory/infectious process resolved. However, as elegantly reviewed elsewhere, alternative hypotheses can be envisaged [12] and further studies are needed to clarify mechanism(s) responsible for lower ADMA levels during acute inflammation/infection.

The role of NO in the pathophysiology of inflammation and infection still remains controversial. Being a vasodilator, NO is implicated in hypotension and septicemic shock. On the other hand, NO has pro-inflammatory and anti-inflammatory actions, and represents a first line element in antimicrobial host defence. NOs inhibitors have been tested in humans to counter the negative haemodynamic effect of NO in sepsis. In a clinical trial that had to be prematurely interrupted, the non-selective NOs inhibitor, N(G)-methyl-L-arginine hydrochloride, increased mortality despite an amelioration in the haemodynamic status [28]. This observation suggests that, in the acute setting, the protective properties of NO outweigh the risk associated with massive synthesis of this compound. Such an interpretation is also in line with our finding that ADMA suppression at peak inflammation was more pronounced in patients with lower blood pressure, i.e. those where the disease was haemodynamically more severe.

In conclusion, resolution of acute inflammation and accompanying nitro-oxidative stress is characterized by an increase in the plasma concentration of ADMA and this phenomenon is accentuated in patients with relatively lower blood pressure. Our data in humans are in line with an experimental model in the rat and imply that during infectious/inflammatory processes, plasma ADMA suppression may actually serve to stimulate NO synthesis or that plasma ADMA may not reflect the inhibitory potential for NO synthesis of this methylarginine at the cellular level.

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Conflict of interest statement. None declared.

References


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