Unilateral nephrectomy causes an abrupt increase in inflammatory mediators and a simultaneous decrease in plasma ADMA: a study in living kidney donors

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Submitted 1 November 2010; accepted in final form 8 August 2011

Unilateral nephrectomy causes an abrupt increase in inflammatory mediators and a simultaneous decrease in plasma ADMA: a study in living kidney donors. Am J Physiol Renal Physiol 301: F1042–F1046, 2011. First published August 10, 2011; doi:10.1152/ajprenal.00640.2010.—Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthases (NOS). Reducing inducible NOS activity in acute inflammation seems to be desirable. In vitro data show that ADMA increases in response to inflammatory mediators, yet the effect of acute inflammation in vivo is scarcely studied. The aim of the study was to evaluate ADMA plasma levels before, during, and after the acute (nonbacterial) inflammatory-like state. Plasma ADMA, L-arginine, C-reactive protein, and IL-6 were determined in 24 healthy subjects undergoing living related kidney donation before as well as 1, 6, 12, 24, 72, and 168 h thereafter. Six hours after nephrectomy, ADMA levels decreased compared with baseline (0.488 ± 0.075 vs. 0.560 ± 0.060 μmol/l, P < 0.05). This difference became even more marked 24 h after the operation (0.478 ± 0.083 μmol/l, P < 0.01 vs. baseline), when the proinflammatory cytokine IL-6 peaked. Seven days after unilateral nephrectomy, ADMA levels were elevated above baseline (0.63 ± 0.05 μmol/l, P < 0.001 vs. baseline). L-Arginine levels decreased already 1 h after nephrectomy (97.5 ± 22.5 μmol/l, P < 0.01 vs. baseline) and paralleled the change in ADMA thereafter. At the end of the observation period when inflammation markers were regressing, L-arginine levels were significantly elevated above baseline (160.6 ± 25.1 μmol/l, P < 0.001 vs. baseline). In summary, this is the first study showing that both ADMA and L-arginine decrease temporarily after unilateral nephrectomy coinciding with the increase in inflammatory mediators. The L-arginine/ADMA ratio, a surrogate for NO production capacity, was only altered for <24 h.

L-Arginine; sepsis; transplantation

ASymmetric dimethylarginine (ADMA) is a naturally occurring arginine inhibitor that is able to inhibit the activity of all three nitric oxide synthase (NOS) isoforms. While long-term inhibition of endothelial NOS (eNOS) by ADMA with a subsequent decrease in NO production (27) is associated with an increased morbidity and mortality (5, 15, 33) as well as disease progression in different patient populations (25), reducing inducible NOS (iNOS) activity in the case of acute inflammation seems to be desirable. This has lead to the application of synthetic NOS inhibitors in patients with septic shock (30). Teleological reasoning would suggest that the endogenous NOS inhibitor ADMA may serve as a physiological brake on overwhelming iNOS activity (9), thus counteracting the effects of inflammation on the endothelium during inflammatory processes. This idea is supported by human data showing that the infusion of bacterial LPS to young, healthy subjects decreases the L-arginine/ADMA ratio, i.e., NO production capacity (20). Moreover, in vitro data by Leiper and coworkers (23) showed that the enzyme dimethylarginine dimethylaminohydrolase (DDAH; the main regulator of ADMA levels) is impaired in the presence of inflammation. In combination with low L-arginine plasma levels, high ADMA plasma levels decrease systemic hemodynamics and reduce blood flow through the kidney, spleen, and liver (26). These findings are in disagreement with preclinical data in a model of LPS endotoxemia (23) as well as with data from patients in whom inflammation resolved (35). In both studies, ADMA was lower in the state of acute inflammation and increased after the inflammation resolved. This suggests that, in contrast to our teleological reasoning, a fall in ADMA in the state of acute inflammation may actually serve to stimulate NO synthesis. To our knowledge, there are no longitudinal prospective data on ADMA and markers of inflammation before, during, and after an episode of elevated inflammation markers by a primary noninfectious cause like a surgical procedure in healthy subjects. We therefore evaluated the time course of plasma ADMA levels as well as markers of inflammation and renal function in 24 healthy subjects undergoing elective living related kidney donation.

METHODS

Patients and procedures. The study was approved by the local Ethics Committee of Hannover Medical School. All patients gave written informed consent. We studied 24 Caucasian living related kidney donors (5 men/19 women) with a mean age of 55.2 ± 8.3 yr. The mean baseline creatinine level of the patients was 61.88 ± 9.72 μmol/l, and their renal function side distribution (right/left) was 48 ± 3%/52 ± 3% Details of the study population are provided elsewhere (18). Blood samples for measurement of plasma ADMA, L-arginine, C-reactive protein (CRP), and IL-6 as well as routine chemistry were drawn before as well as 1, 6, 12, 24, 72, and 168 h after unilateral nephrectomy. Blood samples were immediately cooled on ice and centrifuged at 1,500 g, 4°C for 10 min. Supernatants were stored in 1-ml aliquots at −80°C until further use. Side distribution of renal function was analyzed by a renal szintigraphy with isotope technique
using $^{99m}$Tc-MAG 3 as part of the routine workup for living kidney donation.

**Measurements and calculation.** Plasma concentrations of ADMA and l-arginine were measured applying a recently developed liquid chromatography-mass spectrometry method described elsewhere (19). IL-6 levels were detected with a solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay (Siemens Healthcare Diagnostics, Eschborn, Germany). All other measurements were done with routine laboratory tests using certified assay methods.

**Statistical analysis.** We used GraphPad Prism 5 for statistical analysis. The normality of data distribution was confirmed with the Shapiro-Wilk test. ANOVA was used to compare the biochemical parameters at the different time points with correction to repeated measures. The significance level was set at $P < 0.05$.

**RESULTS**

Living kidney donation was well tolerated in all subjects. Six hours after nephrectomy, the ADMA levels decreased significantly compared with the baseline values ($0.488 \pm 0.075 \mu\text{mol/l}$ vs. $0.560 \pm 0.060 \mu\text{mol/l}$, $P < 0.05$). This difference became even more marked 24 h after the operation ($0.478 \pm 0.083 \mu\text{mol/l}$, $P < 0.01$ vs. baseline). At the end of the observation period, i.e., 7 days after unilateral nephrectomy, ADMA levels were elevated above baseline ($0.63 \pm 0.05 \mu\text{mol/l}$, $P < 0.001$ vs. baseline) (Fig. 1). The changes in ADMA were paralleled by the changes in l-arginine. Baseline l-arginine levels ($118.0 \pm 29.5 \mu\text{mol/l}$) were significantly different from l-arginine 1 h after unilateral nephrectomy ($97.5 \pm 16.2 \mu\text{mol/l}$, $P < 0.001$ vs. baseline). This difference became most marked 12 h after the operation ($77.1 \pm 16.2 \mu\text{mol/l}$, $P < 0.001$ vs. baseline).

As with ADMA, l-arginine levels at the end of the observation period were significantly elevated above baseline ($160.6 \pm 25.1 \mu\text{mol/l}$, $P < 0.001$ vs. baseline). The l-arginine/ADMA ratio, a surrogate for NO production capacity, was 203 $\pm$ 52 at baseline and did decrease abruptly already 1 h after the operation ($169 \pm 31$ ($P < 0.01$ vs. baseline). One day after nephrectomy, the l-arginine/ADMA ratio was not different from baseline ($194 \pm 35$). Seven days after the operation, the l-arginine/ADMA ratio was increased above baseline (Fig. 1, Table 1).

**Table 1. Creatinine, urea, and l-arginine/ADMA ratio before and after kidney donation of healthy subjects**

|       | Baseline | 1 h    | 6 h   | 12 h  | 24 h  | 72 h  | 168 h 
|-------|----------|--------|-------|-------|-------|-------|-------
| Creatinine, $\mu\text{mol/l}$ | 65.4 $\pm$ 8.4 | 74.2 $\pm$ 13.4* | 88.2 $\pm$ 10.2* | 97.4 $\pm$ 9.7* | 103.5 $\pm$ 11.7* | 106.6 $\pm$ 13.3* | 97.6 $\pm$ 13.3* 
| Urea, mmol/l | 5.0 $\pm$ 1.3 | 4.5 $\pm$ 1.1 | 4.9 $\pm$ 0.9 | 5.4 $\pm$ 0.9 | 5.8 $\pm$ 1.4 | 5.3 $\pm$ 1.7 | 5.9 $\pm$ 1.6 
| l-Arginine/ADMA ratio | 203 $\pm$ 52 | 169 $\pm$ 31* | 180 $\pm$ 27* | 164 $\pm$ 37* | 194 $\pm$ 35 | 234 $\pm$ 34* | 247 $\pm$ 36* 

Values are means $\pm$ SE. ADMA, asymmetric dimethylarginine. *$P < 0.001$ vs. baseline.
The inflammation marker CRP was significantly increased 6 h after the procedure compared with baseline (0.60 ± 1.49 vs. 4.20 ± 3.47 mg/l, \( P < 0.05 \)) and peaked at day 3 after the operation (87.85 ± 29.58 mg/l). As expected, the increase in CRP was preceded by an increase in IL-6, which was already significantly elevated 1 h after unilateral nephrectomy (2.00 ± 0.00 vs. 40.20 ± 15.08 ng/l). On day 7 after the operation, both inflammatory markers were decreasing but still above baseline levels (Fig. 1).

Serum urea increased after unilateral nephrectomy with a peak at 12 and 24 h after the procedure, respectively, but did not significantly change during the observation period (Table 1). As expected, creatinine increased from 65.4 ± 8.4 μmol/l at baseline to 74.2 ± 13.4 μmol/l 1 h after nephrectomy \( (P < 0.01 \) vs. baseline) and remained elevated above baseline for the remainder of the observation period (Table 1).

**DISCUSSION**

The pertinent findings of our study were that 1) ADMA temporarily decreases in the state of acute elevated inflammation markers after unilateral nephrectomy despite a reduction in GFR by 50%, 2) the decrease in ADMA is accompanied by an increase in the proinflammatory cytokine IL-6, 3) resolution of inflammation markers goes along with an increase in ADMA above baseline levels, and 4) the l-arginine/ADMA ratio is kept nearly constant in the state of acute inflammation.

**ADMA temporally decreases during the increase in inflammatory markers.** To our knowledge, this is the first study in humans showing that ADMA decreases temporarily in the state of acute elevated inflammation markers in human beings after unilateral nephrectomy.

Two clinical papers suggested already that ADMA, in contrast to our expectations, might be decreased in the state of acute inflammation. Although O’Dwyer et al. (24) found an increase in ADMA levels in critically ill patients compared with healthy controls, ADMA increased by 12% from day 1 of the intensive care unit stay to day 7 in those patients that survived. Zoccoli and coworkers (35) showed in 17 patients with acute bacterial infection that the resolution of acute inflammation was characterized by a 29% increase in the plasma concentration of ADMA. Both reports could by their very nature not show how ADMA levels had been before the acute inflammation. The inverse relationship of the changes in IL-6 to the changes in ADMA suggests a close association, if not causal relationship, in which the increase in IL-6 precedes the changes in ADMA. Interestingly, a post hoc analysis of a trial in septic patients showed an inverse relationship between ADMA and IL-6 and CRP levels (13). This is distinctly different from the state of chronic inflammation in which ADMA is directly related to inflammatory markers (32). Very recently Blackwell et al. (3) showed that ADMA fell by a median of 31% after elective knee arthroplasty, reaching a nadir on day 2 and recovering to baseline by postoperative day 5. Another possible mechanism for the fall in ADMA after unilateral nephrectomy could be the shutdown of the ADMA synthesis and proteolysis pathways as has been shown in erythrocytes (1).

The results of our in vivo study could also help to elucidate the very interesting results of a preclinical study by Carello et al. (7). Using a rat model, these authors found a sustained fall in ADMA plasma levels to 53% of the baseline level after total nephrectomy. Half of the animals died within 72 h; therefore, long-term effects on ADMA levels were not available (7). In a second set of experiments Carello et al. showed that the ligation of the ureter was also associated with a decrease in ADMA by ~50%. Interestingly, the sham-operated animals in their group also had a marked ADMA decrease 12 h after the procedure, which was, however, not quantified. Also, Nijveldt et al. (22) showed in a rat model of endotoxemia that LPS injection resulted in an 12% lower arterial plasma ADMA concentration compared with control rats. These authors did not report on pre- and post-LPS injection ADMA levels. Last but not least, the decrease in ADMA in acute inflammation could explain rather puzzling old data of our group showing a delayed decrease in ADMA 5 h after hemodialysis, possibly due to subclinical dialyzer membrane-induced inflammation and/or poorer water quality (16).

On the other hand, our in vivo data contradict the expectations from two in vitro studies in which TNF-α incubation of endothelial cells resulted in a marked (>60%) decrease in DDHA activity going along with increased ADMA levels while DDHA expression was not altered (10, 14). Similar findings were obtained in another in vitro model where LPS markedly increased the level of ADMA in cultured medium and decreased DDHA activity in endothelial cells (31).

**Adverse effects of elevated ADMA levels in kidney donors?** Several studies have convincingly shown that elevated ADMA levels predict the progression of renal function deterioration (11, 25). The multiple pathophysiological mechanisms that might explain this epidemiological finding are summarized in a recent review paper (34). As we have no long-term data on ADMA levels in these kidney donors, we cannot exclude that ADMA levels will normalize over time.

We also investigated the possibility that gender aspects might affect the changes in the measured parameters; within the limitation of the small sample size, we did not observe any gender difference.

**Possible mechanisms.** It is known that the activity of DDHA is the main regulator of ADMA plasma levels, as 80% of ADMA is metabolized. Only 20% is excreted via the kidney. Therefore, the temporary decrease in ADMA after removal of one kidney was therefore in contrast to our expectations. The very nature of our study does not enable us to elucidate pathophysiological mechanisms of the biphasic time course of ADMA levels after unilateral nephrectomy. From preclinical models, there is evidence for increased metabolic turnover of ADMA during endotoxemia (23). Interestingly, in that study the increased metabolic turnover of ADMA was not accompanied by an increased renal elimination of ADMA, a finding that would explain that even the removal of one kidney does, in the short run, lead to elevated ADMA levels. Also, the rat data by Carello et al. (7) suggest that despite the removal of (both) kidneys ADMA clearance can persist. However, as ADMA levels are increased above baseline 7 days after nephrectomy, short-term effects might be important.

Ueda and coworkers (29) did report that incubation of cultured rat smooth muscle cells with IL-1β dose dependently stimulated not only iNOS but also DDHA expression and enzyme activity, accompanied by an increase in NO metabolites and by a decrease in ADMA content in the culture media. Hence an acute increase in DDHA activity might be an addi-
tional mechanism leading to the decrease in ADMA levels in our clinical model of a noninfectious inflammatory-like state.

Another possibility would be the increased uptake by the $\gamma^+$ transporter during endotoxemia. This would need to be potent enough to overcome the perioperative liberation of ADMA from erythrocytes, which has been described in the preclinical and clinical setting (1, 2). The $\gamma^+$ transporter is responsible for shuttling cationic amino acids, such as arginine, ornithine, and lysine into endothelial cells (8). In rats it has been shown that the expression of cationic amino acid transporters (CAT) was significantly increased in lung, heart, and kidney tissue by LPS injection (12).

Also, in a recent study by Blackwell et al. (4) it has been shown that ADMA decreased during the first 48 h of acute inflammation after total knee arthroplasty. These findings were not caused by increased metabolism of ADMA but may be reflected by an increased intracellular partitioning, which could be explained by more generalized cellular uptake of amino acids by inducing CATs. Teerlink and coworkers (28) showed that the induction of CATs can be initiated by multiple factors like tissue repair, hormones such as insulin, and acute inflammation itself. Hence the postaggressive metabolism after surgical trauma could lead to an upregulation of CATs and following to decreased levels of ADMA.

1-Arginine decreases after unilateral nephrectomy. The kidney plays a major role in 1-arginine metabolism in three principal ways: arginine synthesis, creatinine synthesis, and arginine reabsorption (6). The marked decrease in 1-arginine after unilateral nephrectomy was not surprising. Nijveldt and coworkers (22) observed a similar finding after LPS injection in the rat. In their study 1-arginine decreased by $\sim$40% compared with baseline (22). In line with that, Zoccali et al. (35) found that after resolution of an acute inflammatory state secondary to infectious processes, 1-arginine increased significantly by $\sim$15%. Part of this can also be explained by the liberation of arginase, as has been described for other invasive procedures (17).

Urea. Although not related to the aspect of acute inflammation, we also report our data on urea as our study is the first one to measure urea immediately after kidney donation. Our data are in line with data from Najarian et al. (21), that measured urea immediately after kidney donation. However, we also report our data on urea as our study is the first one to measure urea immediately after kidney donation.

Limitations of the study. Our single-center study is hypothesis generating in nature, suggesting that in the acute inflammatory-like state with two elevated inflammation markers ADMA levels decrease. The design of our study does not allow elucidating the exact pathophysiological processes that lead to fall in ADMA levels in the situation described but can still be hypothesis generating in nature. Also, we do not have data on urine concentration of the studied compounds.

In summary, the results of our study cast serious doubts on the hypothesis that ADMA is an important player, limiting cytokine-stimulated NO synthesis by iNOS in the state of acute (nonbacterial) inflammation. In contrast to this intriguing hypothesis, ADMA decreases in the setting of an acute inflammatory-like state with increased levels of CRP and IL-6, keeping the 1-arginine/ADMA ratio stable.

GRANTS
J. T. Kielstein is supported by a grant from the Else-Kröner-Fresenius Foundation (P63/06/EKMS 06/03). H. Veldink is supported by the StrucMed program of the Hannover Medical School.

DISCLOSURES
J. T. Kielstein owns and hosts the website www.adma.com.

REFERENCES


