

ORIGINAL ARTICLE

## Removal of asymmetric dimethylarginine during artificial liver support using fractionated plasma separation and adsorption

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### Abstract

**Objective.** Asymmetric dimethylarginine (ADMA) is the most potent endogenous nitric oxide synthase inhibitor. Elevated ADMA levels have been linked to increased mortality in different patient populations. Key regulation of ADMA levels mainly takes place in the liver. Hence, ADMA is elevated in liver disease. There is no specific pharmacological treatment to lower the elevated ADMA levels. Hemodialysis is of limited efficiency in removing ADMA as it is highly protein bound. Prometheus<sup>®</sup> is an extracorporeal liver support system which allows the removal of protein-bound toxins. We assessed the efficiency of the Prometheus<sup>®</sup> system in reducing high ADMA levels in patients with liver failure. **Material and methods.** We studied nine patients with acute-on-chronic liver failure and concomitant renal failure already necessitating hemodialysis. Seven patients needed intensive care treatment. Two consecutive sessions of Prometheus<sup>®</sup> therapy of each 4 h were performed in all patients. ADMA and its structural isomer symmetrical dimethylarginine (SDMA) were determined using liquid chromatography–mass spectrometry. **Results.** ADMA levels correlated to model for end stage liver disease (MELD) score ( $r_s = 0.62$ ;  $p < 0.0001$ ). Before Prometheus<sup>®</sup> was started, levels of ADMA and SDMA were elevated ( $1.36 \pm 0.5 \mu\text{mol/l}$  and  $1.90 \pm 0.4 \mu\text{mol/l}$ , respectively). During Prometheus<sup>®</sup> treatments, plasma levels of ADMA dropped by a mean 25% ( $p < 0.0001$ ) and SDMA levels by 22% ( $p < 0.0001$ ). However, there was a significant rebound of ADMA levels between the two therapy sessions ( $p < 0.01$ ). **Conclusions.** This study shows for the first time that plasma levels of ADMA can be effectively lowered by an artificial liver support system (Prometheus<sup>®</sup>). Effective elimination of ADMA might explain some of the beneficial clinical effects of these systems in patients with liver failure.

**Key Words:** ADMA, arginine, extracorporeal dialysis, fractionated plasma separation and adsorption, liver failure, plasma albumin, prometheus, SDMA

### Introduction

Nitric oxide (NO) is the most potent vasodilator that is synthesized from the amino acid L-arginine. Asymmetric dimethylarginine (ADMA) is a naturally competitive inhibitor of the endogenous nitric oxide synthase (NOS) [1]. An increase in ADMA levels is often observed in subjects with classical or novel cardiovascular risk factors such as hypercholesterolemia,

insulin resistance, diabetes mellitus, hypertension and chronic kidney disease [2,3]. ADMA has also shown to be an excellent predictor of mortality in selected patient populations [4–7] as well as in the general population as recently shown for the Framingham population [8]. Symmetrical dimethylarginine (SDMA) is the structural isomer of ADMA which correlates well with different measures of the glomerular filtration rate [9]. It has no known direct effect on NOS, but interferes

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indirectly with NO synthesis by competing with L-arginine for the  $y^+$ -transporter which shuttles L-arginine into the cells [10]. About 20% of ADMA is excreted unchanged in the urine.

However, the primary route (80%) of ADMA clearance is the enzymatic degradation by dimethylamine dimethylaminohydrolase (DDAH), which converts ADMA to citrulline and dimethylamine. Liver has abundant DDAH and plays an important role in the metabolism of ADMA by taking up large amounts of ADMA from the systemic circulation [11]. Evidence for the role of the liver in the elimination of ADMA is confirmed by studies showing that ADMA is elevated in patients with liver cirrhosis or acute alcoholic steatohepatitis [12] and in patients with hepatorenal syndrome [13]. Moreover, a recent study in patients with hepatitis C virus (HCV) associated liver cirrhosis showed a significant correlation between the invasively measured hepatic venous pressure gradient and ADMA levels in hepatic venous blood [14]. In alcoholic hepatitis, ADMA (and SDMA) outperformed established predictors of survival such as Child-Pugh score and model for end stage liver disease (MELD) score [15].

There is sparse evidence that plasma ADMA levels can be reduced by pharmacotherapy [16] and the successful treatment of high ADMA levels using inhibitors of the renin-angiotensin system was accompanied by a reduction of proteinuria and thus protein turnover as well [17]. Since ADMA has a low molecular weight (about 202 Da), comparable with that of urea (60 Da), renal replacement therapy seems to be the option for removing ADMA, resulting in improvement of organ dysfunction and clinical symptoms. However, clinical studies concerning the impact of hemodialysis on ADMA blood levels indicate that this view is oversimplified. In studies showing a significant decrease of ADMA plasma levels in patients with normal liver function by hemodialysis the reduction ranged between 23% [18] and 65% [19]. By contrast, several studies did not show a significant decrease in ADMA at all, comparing pre- and post-dialysis ADMA levels [20–22], in particular not in patients prone to hypotension [23]. There is evidence suggesting that the dialysance and thus removal of ADMA during regular hemodialysis is hampered by its protein binding nature [21]. However, in this analysis ADMA protein binding, which was roughly 90%, was determined only by using crude methods, i.e. deproteinizing the samples using ultracentrifugation with millipore CL tubes (molecular cut off of 10,000). Interestingly, high ADMA serum levels are inversely associated with low serum albumin levels in proteinuric patients [24]. Some more insight was gained from an analysis by Tsikas and Beckmann who found that

human serum albumin does not contain ADMA [25]. In addition erythrocytes contain protein bound and free ADMA [26] which explains the decrease of the L-arginine/ADMA ratio in the state of hemolysis [27].

New extracorporeal devices have recently been developed to support liver detoxification by different techniques aiming at an effective removal of both water-soluble and protein-bound substances [28]. The Prometheus<sup>®</sup> system (Fresenius Medical Care, Bad Homburg, Germany) is based on the method of fractionated plasma separation and adsorption (FPSA). During the pilot trial with FPSA, we could demonstrate its effectivity in removing both protein-bound and water-soluble toxins in patients with acute-on-chronic liver failure [29]. Therefore, the Prometheus<sup>®</sup> system that is capable of removing protein-bound substances seems to be an ideal method to remove excess ADMA in patients with liver failure [29].

## Methods

This work is a secondary analysis of data obtained during the approval study of the Prometheus<sup>®</sup> system. The study was approved by the Ethics Committee of Hannover Medical School. The Declaration of Helsinki and the rules for Good Clinical Practice were followed. Written informed consent was obtained from all patients or next of kin. Nine patients (five males) with liver failure (two with post-hepatic cirrhosis, two with metabolic liver disease, two with acute alcoholic hepatitis, two with liver failure late after liver transplantation and one with primary biliary cirrhosis) were included in the trial. Their mean age was  $51 \pm 7$  years with an average APACHE II score of  $17 \pm 4$ . Average Child-Pugh score was  $12 \pm 2$  points and average MELD score was  $23 \pm 7$ . Hepatic encephalopathy of stage 2 or more was present in 8/9 patients. Significant amount of ascites was also found in 8/9 patients. Seven patients were treated in the intensive care unit.

All patients had concomitant acute kidney injury and were therefore already undergoing hemodialysis with a double-lumen catheter in place. Treatment with FPSA using the Prometheus<sup>®</sup> device and anticoagulation measures were performed as described before [29]. Instead of their regular hemodialysis sessions, all patients underwent two FPSA sessions of  $4.9 \pm 1.1$  h (mean  $\pm$  SD). The Prometheus<sup>®</sup> device was equipped with two adsorbers (Prometh01<sup>®</sup> and Prometh02<sup>®</sup>) in five patients and with one adsorber (Prometh01<sup>®</sup>) in the other four patients. Median blood flow rate was 193 ml/min. The total processed patient blood volume within each treatment was 56l.

Blood samples for the measurement of ADMA, SDMA and arginine were taken before, during (after 30 and 120 min of treatment) and after (directly afterwards as well as 1 and 3 days later) FPSA therapy. Plasma concentrations of ADMA, L-arginine and SDMA were measured applying a recently developed liquid chromatography–mass spectrometry method described elsewhere [30] with the following specifications: L-arginine: sensitivity 0.4  $\mu\text{mol/l}$  at peak/noise ratio of 3, intra-assay variation 4.5% ( $n = 10$ ), inter-assay variation 4.7% ( $n = 6$ ); ADMA: sensitivity 0.02  $\mu\text{mol/l}$  at peak/noise ratio of 3, intra-assay variation 5.5% ( $n = 10$ ), inter-assay variation 7.7% ( $n = 6$ ); SDMA: sensitivity 0.01  $\mu\text{mol/l}$  at peak/noise ratio of 3, intra-assay variation 3.9% ( $n = 10$ ), inter-assay variation 4.9% ( $n = 6$ ).

Additionally, a variety of clinical (such as hepatic encephalopathy) and biochemical parameters (such as bilirubin and ammonia) were assessed. The SPSS program was used for statistical analysis (version 13.0, SPSS, Chicago, USA). All data are presented as median because of the low patient number. Pre- and post-treatment values were compared using two-tailed *T-test for paired data*. Correlations were assessed with *Pearson's correlation analysis*. A *p*-value of  $<0.05$  was considered as statistically significant.

## Results

Except for one reversible episode of catheter-related septicemia and two clotting events of the secondary circuit, Prometheus<sup>®</sup> therapy was well tolerated. Before Prometheus<sup>®</sup> was started, levels of ADMA and of SDMA were elevated ( $1.36 \pm 0.5 \mu\text{mol/l}$  and  $1.90 \pm 0.4 \mu\text{mol/l}$ , respectively). During Prometheus<sup>®</sup> treatments, plasma levels of ADMA dropped by a mean 25% ( $p < 0.0001$ ) and SDMA levels by 22% ( $p < 0.0001$ ). Likewise, L-arginine levels dropped by 24% ( $p < 0.005$ ). The largest decline of ADMA, SDMA and arginine levels occurred during the first 2 h of treatment (Figure 1). However, there was a significant rebound of ADMA and SDMA concentrations between the two therapy sessions ( $p < 0.01$ ).

ADMA levels correlated with levels of L-arginine ( $r = 0.80$ ;  $p < 0.001$ ) and bilirubin ( $r = 0.69$ ;  $p < 0.05$ ). Furthermore, ADMA levels were positively associated with MELD score ( $r = 0.62$ ;  $p < 0.005$ ) and Child-Pugh score ( $r = 0.64$ ;  $p < 0.05$ ). SDMA levels were correlated with ammonia levels ( $r = 0.73$ ;  $p < 0.02$ ), whereas the L-arginine/ADMA ratio was associated with lactate levels ( $r = 0.74$ ;  $p < 0.03$ ). During therapy, hepatic encephalopathy score improved ( $p < 0.05$ ; Table I). Likewise, serum concentrations

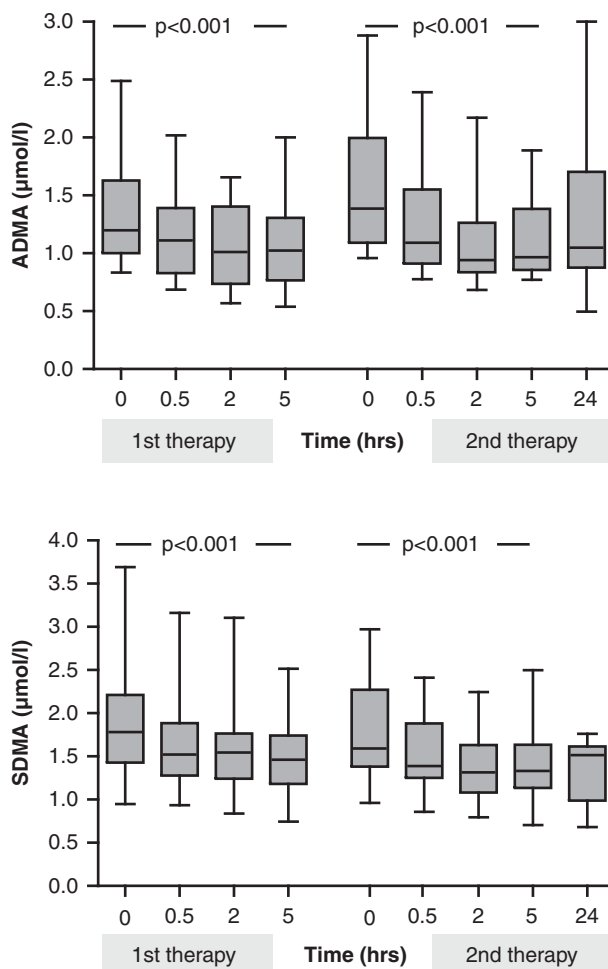


Figure 1. Course of ADMA and SDMA before and after FPSA therapy on two consecutive days of treatment. Data are presented as box plots including median, quartiles and minimum/maximum values.

of protein-bound (e.g. bile acids;  $p < 0.02$ ) and of water-soluble toxins (e.g. urea;  $p < 0.02$ ) declined.

## Discussion

The pertinent findings of our study were that i) ADMA and SDMA were both elevated in patients with acute-on-chronic liver failure; ii) ADMA was related to the severity of the disease assessed by the MELD score; and iii) Prometheus<sup>®</sup>-treatment lowered ADMA significantly.

ADMA is the most potent endogenous NO synthase inhibitor. It exerts powerful biological effects like a decrease in cardiac output and a reduction in renal and cerebral perfusion [31–33], the latter one is also found in patients with liver failure. The liver is the key organ in regulating ADMA levels as it metabolizes most of the ADMA using the enzyme dimethylarginine

Table I. Clinical and biochemical characteristics of study patients.

Parameter	Pre-treatment	Post-treatment	<i>p</i>
APACHE II score	17 ± 4	16 ± 2	0.6
Mean arterial pressure (mmHg)	69 ± 12	62 ± 10	0.1
Heart rate (min <sup>-1</sup> )	93 ± 8	100 ± 8	0.2
Oxygen saturation (%)	97 ± 2	98 ± 2	0.6
Body temperature (°C)	36.6 ± 0.5	36.7 ± 0.7	0.4
Child Pugh score	12 ± 2	11 ± 1	0.5
Hepatic encephalopathy score	2.0 ± 1.1	1.6 ± 1.2	<0.05
Total bilirubin (μmol/l)	395 ± 254	292 ± 183	0.2
Total bile acids (μmol/l)	67 ± 52	43 ± 37	<0.02
Plasma ammonia (μmol/l)	44 ± 27	21 ± 7	0.1
ASAT (U/l)	42 ± 32	46 ± 48	0.9
Gamma-GT (U/l)	57 ± 56	67 ± 68	1.0
Cholinesterase (kU/l)	1.2 ± 0.5	1.6 ± 0.6	0.8
Serum albumin (g/l)	27 ± 4	27 ± 6	0.7
Serum creatinine (μmol/l)	252 ± 138	127 ± 93	0.1
Serum urea (mmol/l)	21.9 ± 7.5	7.8 ± 3.1	<0.02
Venous blood pH	7.41 ± 0.06	7.44 ± 0.05	0.7
Lactate (μmol/l)	1.8 ± 0.8	1.8 ± 0.9	1.0
Serum glucose (mmol/l)	6.4 ± 0.8	6.0 ± 1.4	0.1
Serum sodium (mmol/l)	136 ± 7	134 ± 3	0.8
Serum potassium (mmol/l)	4.0 ± 0.6	4.3 ± 0.4	0.3
Lactate dehydrogenase (U/l)	257 ± 26	242 ± 25	<0.02
C-reactive protein (mg/l)	80 ± 62	67 ± 51	0.7
Leukocytes (thousand/μl)	15.5 ± 12.1	21.6 ± 16.6	0.2
Hemoglobin (g/dl)	9.0 ± 0.5	9.7 ± 1.1	<0.03
Hematocrit (%)	26 ± 2	28 ± 3	<0.02
Platelets (thousand/μl)	53 ± 22	64 ± 29	0.5
Prothrombine time (%)	40 ± 16	41 ± 19	0.7

Abbreviations: ASAT = aspartate aminotransferase.

diaminohydrolase (DDAH) which accounts for about 80% of the ADMA fate. Another 20% is renal excreted.

Several studies have shown that ADMA is elevated in renal failure [3,34] and in patients with impaired liver function ranging from compensated cirrhosis [35] over advanced hepatocellular carcinoma [36] to end stage liver disease [37].

One of the main findings of our study is the fact that ADMA is tightly correlated to established liver disease scores such as the MELD score. To our knowledge, this had not been described before. Indeed, in alcoholic hepatitis it was shown that ADMA is even a better predictor of survival than established risk scores [15]. Furthermore, Vizzutti et al. found a positive correlation between the hepatic venous pressure gradient and ADMA [35].

Interestingly, SDMA levels were correlated with ammonia levels. Currently, SDMA is thought to be an excellent marker of glomerular filtration rate [9] and is also increased in patients with hepatorenal syndrome [13].

Patients with liver disease and renal failure exhibit high levels of ADMA, which are predictive of survival, at least in patients with alcoholic hepatitis [15]. Lowering ADMA either by overexpressing DDAH or

using adenoviral gene transfer has been shown to alleviate cardiovascular pathology [38] and to prevent deterioration of renal function [39] in preclinical models. Hence based on this pathophysiological evidence, lowering ADMA seems to be associated with favorable effects. Both dimethylarginines, ADMA and SDMA, were reduced by the Prometheus<sup>®</sup> treatment when comparing pre- and post-treatment serum levels. However, the extent of reduction was lower than we expected. Prometheus<sup>®</sup> therapy did not bring down ADMA levels to normal or near-normal levels which, however, may be related to the low number of treatment sessions. Dialysis treatment alone was by some authors reported to lower ADMA levels by up to 65% whereas others did not find a reduction by dialysis at all as recently reviewed by Jacobi et al. [34]. In an earlier study we found about 37 μmol, i.e. 12% of the ADMA produced per day in the collected dialysate after a regular high flux hemodialysis session [21]. This is in line with a study by Achan et al. who found that in healthy men only 50 μmol per day are excreted via renal excretion [40]. The poor dialysance of ADMA, which has universally shown to be much less than the dialysance of urea, is currently thought to be caused by the significant protein binding of

ADMA. For that reason we assumed that the Prometheus<sup>®</sup> treatment would be more efficient in reducing ADMA levels. However, there are several reasons why this might not be the case. Probably the most important reason for the low efficiency of ADMA and SDMA clearance by Prometheus<sup>®</sup> therapy is the distinct rebound of the cleared substances after treatment. This effect has been described for many other substances and is even statistically significant in this study [41,42]. In general, a high rate of generation and redistribution from the tissue is thought to be responsible for the rebound effect. Moreover, lack of ADMA metabolizing capacity, i.e. low DDAH activity and the absence of renal excretion due to acute kidney injury will contribute to the observed rebound of ADMA and SDMA. Hypothetically, besides plain removal, the Prometheus<sup>®</sup> treatment could have altered the activity of DDAH, the enzyme hydrolyzing about 80% of the daily ADMA production. Investigating this rather complex scenario of oxidative stress, inflammatory cytokines and pH changes, which can all influence ADMA activity, is however beyond the scope of this study. There might be a potential harmful effect of this rebound of plasma ADMA levels. Previous studies on ADMA infusion in healthy men show that even small amount of exogenous ADMA decrease renal plasma flow [32] and increase sodium retention [43], both known to be harmful to patients with liver failure.

Moreover, our study has several limitations. We did not investigate the effect of the dialysis part alone in these patients, hence we are unable to quantitatively assess to what degree the albumin absorption contributed to the ADMA clearance. We also did neither analyze the whole spent dialysate nor measured the ADMA content in the albumin cartridges.

In conclusion, we could demonstrate for the first time that plasma levels of ADMA can be effectively lowered by an artificial liver support system (Prometheus<sup>®</sup>). However, an important rebound effect takes place after therapy. Nevertheless, the elimination of ADMA could be involved in positive clinical effects of these systems in patients with liver failure.

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