Concentration of dimethyl-l-arginine in the plasma of patients with end-stage renal failure

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Abstract. Dimethyl-L-arginine (asymmetric dimethyl-L-arginine; ADMA) and Dimethyl-L-arginine (symmetric dimethyl-L-arginine; SDMA) are naturally occurring analogues of L-arginine, the substrate for nitric oxide (NO) synthesis. ADMA is a potent inhibitor of NO synthesis, and accumulates in the plasma of patients with renal failure. However the precise concentration of ADMA and SDMA in renal patients is still controversial. This study was performed to measure plasma ADMA and SDMA concentrations by two different HPLC techniques in nine healthy controls and 10 uraemic subjects, and to investigate the effects of haemodialysis. In controls, the mean (±SEM) plasma concentrations of ADMA and SDMA were 0.36 ± 0.09 and 0.39 ± 0.05 μmol/l respectively, yielding an ADMA/SDMA ratio of 1.2 ± 0.17. In uraemic patients, the plasma concentrations of ADMA and SDMA were 0.9 ± 0.08 μmol/l (P < 0.001 compared to controls) and 3.4 ± 0.3 μmol/l (P < 0.001 compared to controls) with an ADMA/SDMA ratio of 0.27 ± 0.015 (P < 0.001). In the course of one 4 h haemodialysis session, ADMA concentrations decreased from 0.99 ± 0.13 to 0.77 ± 0.3 μmol/l and SDMA concentrations from 3.38 ± 0.44 to 2.27 ± 0.21 μmol/l. The plasma ADMA/creatinine ratio tended to increase from 1.26 ± 0.20 × 10⁻³ to 2.01 ± 0.41 × 10⁻³. It is concluded that there is a modest (3-fold) but definite increase in plasma ADMA concentration in uraemic patients compared to controls. SDMA accumulates to a greater degree (8-fold increase) and more closely parallels creatinine concentration than ADMA. The change in the ADMA/SDMA ratio is not accounted for by greater renal or dialysis clearance of ADMA, and, even though alternative explanations are not excluded, greater metabolism of ADMA than SDMA is the most likely explanation. Although small in magnitude, the increase in ADMA concentration might be biologically significant.

Key words: uraemia; dialysis; NO synthase; ADMA; SDMA

Introduction

ADMA and Dimethyl-L-arginine (asymmetric dimethyl-L-arginine; ADMA) and Dimethyl-L-arginine (symmetric dimethyl-L-arginine; SDMA) accumulate in the plasma of patients with renal failure, and of these, ADMA is a potent inhibitor of nitric oxide (NO) synthase (NOS) [1]. This guanidine compound inhibits each of the three isoforms of NOS (endothelial, neuronal and macrophage). It accumulates in the plasma of patients with renal failure and might contribute to certain pathophysiological features of this condition. In the initial study [1], ADMA was measured using a non-derivatized HPLC method. More recently, Anderstamm et al. [2], using a derivatized method, confirmed plasma ADMA concentration to be elevated in dialysed patients and decreased by dialysis, although the reported concentrations were lower than those described previously [1]. Whilst Arese et al. [3] reported that the majority of plasma samples of dialysed patients inhibited each isoform of NOS in vitro, about 20% of samples actually stimulated NOS. Noris et al. [4] also suggested the presence in plasma of factors that enhanced conversion of L-arginine to L-citrulline in human umbilical vein endothelial cells, and there are also preliminary reports of increased NO production during dialysis, as assessed by NO measurements in expiratory air [5]. Increased NO production was related to intradialytic hypotension [6] and was reproduced in vitro by blood–dialysis membrane interaction [7]. The issue is further complicated by the heterogeneity of guanidine compounds that accumulate in the plasma of uraemic patients, certain of which (including methyl-guanidine) might also inhibit NOS [8]. Because of these discrepancies in the literature concerning the precise concentration of methylated derivatives of L-arginine in renal failure, and because of the considerable biological relevance of this issue,
we measured ADMA and SDMA in patients with end-stage renal failure about to start dialysis, patients established on dialysis (before, during and after one dialysis session) and healthy controls. Plasma samples were analysed using two different methods, one identical to that described previously [1] and a novel method (in which methylated arginines were derivatized) developed for the measurement of the related methylarginine (N\(^{\text{Me}}\)monomethyl-L-arginine; L-NMMA) that is in clinical trials for the management of septic shock.

**Subjects and methods**

**Design of the study**

We studied three groups of individuals: nine healthy subjects (three female, six male, mean age 24 ± 4 years), four subjects (two male, two female, mean age 64 ± 4.9 years) with end-stage renal failure who were being prepared to start dialysis (S-creatinine > 500 μmol/l), and six dialysis patients (four male, two female, median age 66.5 ± 7 years). The latter patients had been maintained on haemodialysis for a median of 2.0 years (range 1–7) and were studied during one 4 h dialysis session (measured blood flow 200 ml/min; polysulphone dialyser F8 (1.8 m² surface); dialysate flow 0.5 l/min; dialysate with 32 mmol/l HCO\(_3\); Braun Secura HD machine). Heparinized blood samples were drawn at times 0, 1, 2 and 4 h after beginning of dialysis, and 0.5, 1, 2, 3 and 6 h following dialysis. Forty-four hour urine samples were collected in the four pre-dialysis patients and on the day prior to dialysis in three uraemic patients with residual urine output (three patients on dialysis were anuric).

**HPLC methods**

**Non-derivatized method.** Samples were purified by sequential passage through Bond Elut SCX and CBA columns (Jones Chromatography Ltd, Hengoed, Mid Glamorgan, Wales, UK), and eluted with ammonia/methanol [1]. Separation of amino acids was achieved on an ODS C\(_{18}\) HPLC column (Phase Separation, Queensferry, Wales, UK) using a mobile phase containing 0.025 mol/l phosphoric acid (pH 5.0), 0.01 mol/l hexane sulphonic acid (Romil Laboratories, Loughborough, UK) and 1% (v/v) acetonitrile (Romil Laboratories). Flow was maintained at 1 ml/min and amino acids were detected by UV absorbance at 200 nm.

**Derivatized method.** Samples were purified by centrifugation (Millipore Ultrafree MC 5 kDa filters) and derivatized using o-phthalaldehyde [9]. Separation of amino acids was achieved on an SGE C\(_{18}\) HPLC column by gradient elution with acetonitrile, the second solvent containing ammonium acetate (25 mmol/l), citric acid (10 mmol/l) and potassium chloride (330 mmol/l; pH 5.5). Derivatized amino acids were detected by electrochemical oxidation [9].

ADMA and SDMA concentrations were determined by reference to synthetic standards.

**Statistics**

Data are expressed as means ± SEM. Comparisons were made using Wilcoxon's test for paired or unpaired samples or by analysis of variance (ANOVA) as appropriate.

**Results**

**Concentration of ADMA and SDMA**

The concentrations of ADMA, SDMA and \(L\)-arginine in the plasma of healthy subjects (determined by the non-derivatized HPLC method) were 0.36 ± 0.09, 0.39 ± 0.05 and 75.3 ± 13 μmol/l, respectively (n = 9). The mean ratios of ADMA to SDMA and ADMA to \(L\)-arginine were 1.2 ± 0.17 and 0.007 ± 0.001, respectively (n = 9). In patients with end-stage renal failure, the concentrations of ADMA and SDMA were higher (0.9 ± 0.08 and 3.4 ± 0.03 μmol/l, respectively; P < 0.001; n = 10), but ADMA concentration was not significantly different (35.4 ± 3.1 μmol/l; P < 0.8; n = 4). Subgroup analysis of uraemic plasma showed that the ADMA concentrations were similar in anuric patients (1.0 ± 0.23 μmol/l; n = 3), in patients with residual urine output (0.98 ± 0.18 μmol/l; n = 3) and in pre-dialysis patients (0.77 ± 0.08 μmol/l; n = 4; P > 0.05 by ANOVA).

The plasma ADMA/SDMA ratio was reduced (0.27 ± 0.015; P < 0.001; n = 10) and the ADMA/\(L\)-arginine ratio was increased (0.02 ± 0.001; P < 0.01; n = 4). The clearance of ADMA, SDMA and creatinine in patients with renal failure was similar (5.7 ± 2.8, 5.0 ± 2.6 and 5.1 ± 1.3 ml/min, respectively; P > 0.5; n = 4). Subgroup analysis of the ADMA/SDMA ratios in uraemia revealed no differences between anuric patients (0.29 ± 0.04; n = 3), patients with residual urine output (0.27 ± 0.04; n = 3) or pre-dialysis patients (0.24 ± 0.025; n = 4; P > 0.5 by ANOVA).

In three subjects the ADMA concentrations determined by the non-derivatized and derivatized HPLC method were (respectively) 0.6 ± 0.05 and 0.4 ± 0.05 μmol/l (P < 0.05), the SDMA concentrations were 1.4 ± 0.2 and 1.3 ± 0.3 μmol/l (P > 0.1) and the ADMA/SDMA ratios were 0.42 ± 0.06 and 0.35 ± 0.08 (P > 0.1) (Fig. 1).

**Change of plasma ADMA and SDMA concentrations during one 4 h haemodialysis session (Table 1 and Figs 2 and 3)**

As shown in Table 1, a modest decrease of ADMA concentration was seen at the end of the 4 h dialysis session, but this was not statistically significant. There was, however, a marked and significant increase in the plasma ADMA/creatinine and SDMA/creatinine ratios. There was a rapid rebound of plasma ADMA and SDMA concentrations after dialysis.

**Discussion**

The results of this study demonstrate that in uraemia, the plasma concentrations of ADMA and SDMA are higher than in controls. This observation is consistent with previous reports [1], although the levels found in the present study are somewhat lower. Dialysis was associated with a modest decrease in the total concentration of dimethylarginines. The concentrations of
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Fig. 1. Chromatogram of human plasma. Non-derivatized amino acids are detected by UV absorbance at 200 nm. The amounts of arginine, ADMA and SDMA are derived from the area under the chromatogram peaks with reference to synthetic standards; in this chromatogram of healthy human plasma, the areas under the ADMA and SDMA peaks are similar.

Fig. 2. Molar ADMA/creatinine and SDMA/creatinine ratios in six haemodialysis patients before and after one 4 h haemodialysis.

ADMA and SDMA pre- and during dialysis are consistent with those reported by Arese et al. [3]. In view of the controversy concerning the absolute values [2] it is of note that, in this study, the values obtained with the original HPLC method from partially purified plasma [1] were largely confirmed in this study by an independent derivatized method, developed to measure methylarginines for drug regulatory purposes.

SDMA accumulated to a greater extent (8-fold) in uraemia than ADMA (3-fold), and this is reflected by the significantly lower plasma ADMA to SDMA ratio in renal patients compared to controls. The change in ADMA/SDMA ratio is not accounted for by differences in the renal clearance of the two enantiomers, since this ratio was similar in patients with renal failure not on dialysis, and the clearance of ADMA and SDMA by dialysis appeared to be similar. Although our data do not exclude the possibility that more SDMA is synthesized, the most likely explanation is selective metabolism of ADMA but not SDMA. Indeed the enzyme dimethylarginine dimethylaminohydrolase (DDAH) that metabolizes ADMA but not SDMA is found in a variety of human tissues, including kidney, pancreas and human blood vessels [10,11]. Consequently, plasma concentrations of ADMA are
Dimethylarginines accumulate in renal disease and such increase is cross-validated by comparison of two different analytical methods.  

As an NOS inhibitor, endogenous ADMA might contribute to the pathophysiology of renal dysfunction and complications of renal failure.  

The more marked accumulation of SDMA compared to ADMA points to a potential role for DDAH in maintaining low concentrations of ADMA in renal failure.  

The latter aspect warrants further investigation.

**References**