Brief Report

Net renal extraction of asymmetrical (ADMA) and symmetrical (SDMA) dimethylarginine in fasting humans

Robert J. Nijveldt¹,*, Paul A. M. van Leeuwen¹, Coen van Guldener²,4, Coen D. A. Stehouwer², Jan A. Rauwerda¹ and Tom Teerlink³

Departments of ¹Surgery, ²Internal Medicine and ³Clinical Chemistry, VU University Medical Center, Amsterdam and ⁴Department of Internal Medicine, Amphia Hospital (Langendijk), Breda, The Netherlands

Abstract

Background. Recently, the potential importance of dimethylarginines as endogenously produced inhibitors of nitric oxide synthase has become clearer. Interestingly, elevated levels have been reported in patients with vascular disease, but especially in patients suffering end-stage renal disease. Although the kidney obviously seems to play a key role in the elimination of dimethylarginines, clear insight into the renal handling of these compounds is lacking. Thus, our aim was to investigate the renal extraction of dimethylarginines.

Methods. Plasma concentrations of dimethylarginines were determined in both arterial and renal venous blood in 20 fasting patients with normal renal function. Renal extraction was calculated as the arteriovenous concentration difference divided by the arterial concentration times 100%.

Results. A significant renal extraction was found for both dimethylarginines. Renal extraction was significantly higher for asymmetrical dimethylarginine (ADMA) when compared with symmetrical dimethylarginine (SDMA) (16.2 vs 10.5% respectively, P < 0.001). In addition, arterial SDMA concentration, but not ADMA concentration, significantly correlated with arterial creatinine concentration.

Conclusions. In healthy humans, the kidney contributes to the regulation of plasma levels of dimethylarginines, since both ADMA and SDMA were significantly extracted from the arterial supply. Interestingly, a higher renal extraction of ADMA was found when compared to SDMA extraction, which strongly suggests the presence of an additional catabolic pathway for ADMA in the kidney.

Keywords: L-arginine; nitric oxide; nitric oxide synthase; renal failure

Introduction

Abnormalities of nitric oxide (NO) production have been implicated in many diseases including hypertension, pre-eclampsia, multiple sclerosis, septic shock, renal failure, hypercholesterolaemia and atherosclerosis. The importance of dimethylarginines as endogenous inhibitors of the arginine–NO pathway is emerging [1]. ADMA acts directly on the enzyme NO synthase, while its analogue, SDMA, is probably important as a competitive inhibitor for arginine transport across cell membranes [2].

In 1992, Vallance et al. [3] reported elevated levels of ADMA in patients with renal failure, and elevated levels of dimethylarginines may be responsible for the hypertension seen in patients with end-stage renal disease. Recently, Zoccali et al. [4] studied the relationship between cardiovascular risk factors and plasma ADMA concentration in a cohort of 225 haemodialysis patients, and found that plasma ADMA is a strong and independent risk factor of overall mortality and cardiovascular outcome. Additionally, in patients with chronic renal failure, a sharp rise in SDMA, the stereo-isomer of ADMA, has been reported [5]. Although SDMA has no inhibitory activity towards NO synthase, Fleck et al. [5] pointed out the potential importance of SDMA, and in their study in a large population of renal-failure patients, they concluded that not only ADMA levels but also SDMA levels were probably responsible for hypertension, possibly by competition for reabsorption between SDMA and arginine in the kidney.

Although the kidney obviously seems to play a crucial role in the regulation of plasma concentrations of ADMA and SDMA, the extent of the kidney’s contribution to the elimination of dimethylarginines has...
never been investigated. In the past, several groups have demonstrated that the human kidney is capable of excreting both ADMA and SDMA [6,7]. However, from these data, no conclusions could be drawn on the net renal extraction of dimethylarginine, because not only urinary excretion but also metabolic pathways within the kidney seem to determine renal dimethylarginine handling. Especially for ADMA a main metabolic pathway has been described, namely degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), and a high DDAH activity has been observed in the kidney [8–10].

By calculation of the renal extraction rate from concentrations of dimethylarginines in arterial and renal venous blood samples, an accurate measure of the net contribution of the kidney in the maintenance of systemic dimethylarginine concentration is obtained.

Accordingly, we aimed to quantify renal extraction of dimethylarginines in humans with normal renal function by measuring concentrations of dimethylarginines in aorta and renal venous blood.

Subjects and methods

Patients and experimental design

Twenty patients (13 men) with normal renal function, aged 63.8 ±10.7 (SD) years, undergoing elective cardiac catheterization for the assessment of coronary artery disease, participated in the study. The study was approved by the hospital ethics committee. After being informed about the nature, purpose and risks of the procedure, all patients gave their voluntary consent. Previously, our group had demonstrated that the human kidney is capable of excreting both ADMA and SDMA [6,7]. However, from these data, no conclusions could be drawn on the net renal extraction of dimethylarginine, because not only urinary excretion but also metabolic pathways within the kidney seem to determine renal dimethylarginine handling. Especially for ADMA a main metabolic pathway has been described, namely degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), and a high DDAH activity has been observed in the kidney [8–10].

By calculation of the renal extraction rate from concentrations of dimethylarginines in arterial and renal venous blood samples, an accurate measure of the net contribution of the kidney in the maintenance of systemic dimethylarginine concentration is obtained.

Accordingly, we aimed to quantify renal extraction of dimethylarginines in humans with normal renal function by measuring concentrations of dimethylarginines in aorta and renal venous blood.

Plasma analysis of AMDA, SDMA, arginine and creatinine

Arginine, ADMA and SDMA were measured simultaneously by high-performance liquid chromatography with fluorescence detection [12]. Briefly, 0.1 ml of plasma was mixed with 0.1 ml of a 40 μmol/l solution of the internal standard L-NMMA and 0.8 ml phosphate-buffered saline (PBS). This mixture was applied to Oasis MCX solid-phase extraction columns (Waters) for extraction of basic amino acids. The columns were consecutively washed with 1.0 ml of 100 mmol/l HCl and 1.0 ml methanol. Analytes were eluted with 1.0 ml of concentrated ammonia/water/methanol (10/40/50). After evaporation of the solvent under nitrogen, the amino acids were derivatized with orthophthalaldialdehyde reagent containing 3-mercaptopropionic acids. The derivatives were separated by isocratic reversed-phase chromatography on a Symmetry C18 column (3.9 x 150 mm; 5-μm particle size; Waters). Potassium phosphate buffer (50 mmol/l, pH 6.5), containing 8.7% acetonitrile was used as mobile phase at a flow-rate of 1.1 ml/min and a column temperature of 30 °C. Fluorescence detection was performed at excitation and emission wavelengths of 340 and 455 nm, respectively. After elution of the last analyte, strongly retained compounds were quickly eluted by a strong solvent flush with 50% acetonitrile, resulting in a total analysis time of 30 min. The intra-assay CVs for arginine, ADMA and SDMA were 0.4, 1.2 and 0.8%, respectively. The interassay CVs for arginine, ADMA and SDMA were 2.9, 2.0 and 2.6%, respectively. Serum creatinine was measured by a modified Jaffé method.

Calculations and statistical methods

Renal extraction (RE) is calculated as \([A–V]/[A]\) times 100%, where [A] and [V] denote arterial and renal venous plasma concentration, respectively. RE was considered significant if the 95% confidence interval did not include zero. Comparison of kidney handling of ADMA and SDMA was performed by non-parametric Wilcoxon signed-rank test. Correlations between plasma concentrations of dimethylarginines and creatinine levels were evaluated by a non-parametric Spearman test.

Statistical analysis was performed using the SPSS 9.0 statistical package. Values are expressed as mean ± SE, and \(P<0.05\) was considered statistically significant.

Results

Patients had normal kidney function, as indicated by the arterial creatinine concentration (90.5 ± 3.5 μmol/l). Arteriovenous concentration differences and renal extraction (RE) of ADMA, SDMA, arginine, and creatinine are presented in Table 1. A significant net RE was found for ADMA, SDMA and creatinine, whereas a net release was observed for arginine. Renal extraction was significantly higher for ADMA when compared with SDMA (\(P=0.001\)).
Arterial ADMA concentration did not correlate with arterial creatinine levels (Figure 1), whereas a significant correlation was found between arterial SDMA concentration and arterial creatinine concentration (Figure 2).

**Discussion**

The present study clearly demonstrates, by determination of the net RE, the important role of the kidney in the elimination of dimethylarginines in fasting humans.

Recently, the potential importance of dimethylarginines as endogenously produced inhibitors of NO synthase has become clearer, as elevated levels of ADMA have been correlated with impaired endothelial function as measured by endothelium-dependent vasodilatation [3]. Plasma levels of ADMA have been found to be elevated in patients with vascular disease, pre-eclampsia, essential hypertension and heart failure. Until now, however, most studies in the field of dimethylarginines focused on the elevation of ADMA and SDMA in renal failure [3,4], and it is generally accepted that accumulation of dimethylarginines is related to a decreased renal elimination capacity.

McDermott [13] revealed that urinary excretion was the main elimination route for SDMA in rabbits, whereas ADMA was partly eliminated by other metabolic pathways. In the rat, Ogawa et al. [14] investigated the metabolic fate of ADMA and SDMA isotopically, and found that 4.6% of injected ADMA was found in the first 12-h urine as unchanged ADMA, compared to 17.8% for SDMA. Their study provided strong evidence for the existence of an additional pathway for the elimination of ADMA, leading to the formation of citrulline and related amino acids. This pathway seemed to be the main route for ADMA elimination, as most ADMA-derived radioactivity was found in tissues instead of urine. Later this catalytic pathway was recognized in both rats and humans and proved to be degradation by the enzyme DDAH [15]. In both humans and rats, a high DDAH activity has been observed in the kidney [8–10]. The results of our study are in concordance with these previous reports, as renal ADMA elimination was found to be significantly higher than SDMA elimination, strongly suggesting the presence of an additional metabolic pathway for ADMA.

Al Banchaabouchi et al. [7] investigated the relationship between plasma levels and urinary concentrations of ADMA and SDMA, and calculated clearances of dimethylarginines and fractional excretion rates (clearance of dimethylarginines divided by the clearance of creatinine times 100%) in healthy humans. They found similar fractional excretion rates for ADMA and SDMA (68.2 and 71%, respectively), and it was calculated that ≈ 30% of both dimethylarginines was reabsorbed. However, their study was limited by the fact that no arteriovenous concentration differences were measured and no net RE could be measured, making it impossible to calculate true reabsorption rates. If the metabolism of dimethylarginines were negligible, we should have found ~ 30% lower RE rates for ADMA and SDMA when compared with the renal creatinine extraction, caused by reabsorption. Indeed, in the case of SDMA, this statement holds true, and therefore metabolism is probably not substantial. However, RE of ADMA was significantly higher, and almost equalled creatinine extraction, which further supports the presence of metabolism of ADMA within the kidney. Demonstrating a significant correlation between arterial SDMA and creatinine concentrations, but not between ADMA and creatinine, corroborates this finding. Thus, SDMA greatly depends on excretory renal function, while for ADMA, other eliminatory processes are also important.
Interestingly, in humans with end-stage renal failure, the rise of SDMA was more pronounced than the rise of ADMA [5]. In our study we did not investigate RE in renal failure patients, and therefore cannot say whether both renal excretion and metabolic degradation within the kidney are equally impaired in end-stage renal failure. However, at least in extra-renal tissues, catalytic degradation of ADMA may be preserved during renal dysfunction, thereby explaining the more pronounced elevation of SDMA.

Although serum creatinine level was normal in all patients, slight alterations in renal function may have been present due to diffusely atherosclerotic disease and/or medication (low aspirin and β-blockers). Our data relate therefore to a normal to mildly impaired renal function.

It should be emphasized that the fact that we found significant RE for both dimethylarginines does not mean that the kidney does not produce these substances itself. Our data merely reflect that net uptake by the kidney occurs.

Patients in the present study were in the fasted state, and it remains unknown whether a change in dimethylarginine elimination occurs under non-fasting conditions. However, Kakimoto et al. [16] reported fairly constant dimethylarginines in urine specimens of human subjects, indicating at least that urinary excretion is not significantly influenced by diet. The origin of the compounds seems to be endogenous.

By using our HPLC method, simultaneous measurement of arginine concentrations was performed. Although arteriovenous concentration differences and arginine handling by the kidney has been studied extensively in the past by our group and others [11,17,18], we chose to present data on arginine, as arginine is the key amino acid in the arginine–NO pathway, and its molecular structure and biological behaviour closely resembles that of the dimethylarginines. In addition, it has been shown that cellular uptake of these cationic amino acids is mediated by the same cellular amino acid transporters [19]. In the present study, a significant net release of arginine was found, which is in agreement with previous reports.

RE of creatinine was slightly lower than 20% because the Jaffé method was used. In serum, a heterogeneous group of substances, named ‘chromogens’, interfere with the colorimetric Jaffé reaction. As these chromogens are not extracted by the kidney, this results in an apparent reduction of the RE of creatinine [20].

We conclude that in healthy humans the kidney contributes to the regulation of plasma levels of dimethylarginines, since both ADMA and SDMA were significantly extracted from the arterial supply. Interestingly, a higher RE of ADMA was found when compared with SDMA extraction, which strongly suggests the presence of an additional catabolic pathway for ADMA in the kidney.

References

5. Fleck C, Janz A, Schweitzer F et al. Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in renal failure patients. Kidney Int 2001; 59 [Suppl 78]: S14–S18
17. Prins HA, Houdeik AP, Wijzer MJ et al. Reduced arginine plasma levels are the drive for arginine production by the kidney in the rat. Shock 1999; 11: 199–204

Received for publication: 27.2.02
Accepted in revised form: 4.7.02