Asymmetrical Dimethylarginine Plasma Concentrations Are Related to Basal Nitric Oxide Release but Not Endothelium-Dependent Vasodilation of Resistance Arteries in Peritoneal Dialysis Patients

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Vascular dysfunction in chronic renal failure may be linked to reduced nitric oxide (NO) bioactivity and increased circulating concentrations of the endogenous NO synthase inhibitor asymmetrical dimethyl l-arginine (ADMA). The association between ADMA and basal endothelial NO release and endothelium-dependent vasodilation in resistance arteries of chronic renal failure patients is unknown. Forearm blood flow responses to the endothelium-dependent vasodilator acetylcholine, the endothelium-independent vasodilator nitroglycerine, and the endothelium-dependent vasoconstrictor N(G)-monomethyl-L-arginine (l-NMMA) were assessed in 37 peritoneal dialysis patients. l-arginine and ADMA plasma concentrations were measured by HPLC. ADMA (mean ± SEM: 0.68 ± 0.02 μmol/L) was associated with basal forearm blood flow (r = −0.33; P < 0.05) and l-NMMA induced vasoconstriction (r = −0.55; P < 0.0005), but not with dilator effects of acetylcholine or nitroglycerine. l-arginine (68 ± 3 μmol/L) tended to correlate with acetylcholine-induced vasodilation (r = 0.32; P = 0.05) but was not associated with other parameters. ADMA is related to basal but not to acetylcholine-stimulated NO bioactivity in patients on peritoneal dialysis. Impaired endothelium-dependent vasodilation found in chronic renal failure is not explained by elevated circulating NO synthase inhibitors in renal failure.


Endothelial dysfunction is characterized by reduced bioactivity of the antiatherogenic molecule nitric oxide (NO) and is considered a proatherogenic condition (1). Chronic renal failure is associated with impaired endothelial function (2). This may contribute to the high cardiovascular mortality seen in these patients. Increased concentrations of the endogenous competitive NO synthase inhibitor asymmetrical dimethyl l-arginine (ADMA) could be related to endothelial dysfunction (3). ADMA is elevated in patients with chronic renal failure, partly because of reduced renal excretion (4), and is an independent predictor of cardiovascular events in hemodialysis patients (5). Thus ADMA may be regarded as a uremic toxin (6).

The capacity of human endothelium to release NO can be assessed in vivo with different methods. Two methods have widely been applied. The invasive forearm blood flow technique uses infusion of vasoactive substances into the brachial artery to provoke endothelium-dependent vasodilation or vasoconstriction of forearm resistance arteries. The increase or decrease of blood flow is usually determined by venous occlusion plethysmography and related to endothelial function (7). The second method measures flow-mediated dilation of the brachial artery (FMD) in response to reactive hyperemia after occlusion of the forearm circulation with high-resolution ultrasound. This technique evaluates a surrogate parameter for shear stress–induced activation of endothelial NO synthases in a large conduit artery (8). Pharmacologic doses of ADMA cause vasoconstriction in healthy humans (9). Consistently, an inverse relationship between ADMA and endothelium-dependent vasodilation measured by FMD has been reported (10–13). Despite the fact that endothelial dysfunction has been detected in similar disease conditions with both methods (7,14 to 17), measurement of FMD has little if any correlation with endothelium-dependent vasodilation assessed with the forearm blood flow technique (18,19). Thus, the impact of ADMA elevation on resistance artery reactivity as assessed with the invasive forearm blood flow technique cannot be extrapolated from FMD studies. The association between ADMA and endothelium-dependent vasodilation, as well as basal endothelial NO release in resistance arteries, has not been studied yet.

We have therefore tested the hypothesis that ADMA concentrations in chronic renal failure are associated with reactivity of the forearm vasculature. Forearm blood flow was measured in response to infusions of the endothelium-dependent vasodilator acetylcholine and the endothelium-independent vasodilator...
nitroglycerine into the brachial artery. Basal endothelial NO formation was assessed as reactivity to intraarterial N(G)-monomethyl-l-arginine (l-NMMA), a competitive NO synthase antagonist.

Materials and Methods

The study protocol was approved by the Ethics Committee of the Medical University Vienna and conforms with the principles outlined in the Declaration of Helsinki, including current revisions and the European Guidelines on Good Clinical Practice.

Subjects

Thirty-seven patients with chronic renal failure who were treated with continuous ambulatory (n = 15) or automated (n = 22) peritoneal dialysis (PD) at the Medical University Vienna were included in the study after written informed consent was obtained. All patients had been stable on PD for at least 3 mo and were without episodes of peritonitis for at least 2 mo before inclusion. Exclusion criteria were acute infection, acute vascular disease (history of stroke, myocardial infarction, or peripheral arterial occlusive disease within 3 mo before the study), pregnancy, and participation in another trial within 4 wk before the study day. Coronary artery disease was defined as history of percutaneous or surgical revascularization and/or myocardial infarction. Cerebrovascular arterial disease was defined as history of percutaneous or surgical revascularization and/or stroke. No patient had peripheral arterial occlusive disease. Hypertension was defined as BP >140/90 mmHg and/or use of antihypertensive medication. Detailed patient characteristics and plasma and urine parameters are presented in Table 1. Smokers did not smoke for 12 h before nor during the study.

Measurement of Endothelial Function

Forearm blood flow was measured in both arms as described previously (20,21). Briefly, strain gauges, placed on the forearms, were connected to plethysmographs (EC-6, DE Hokanson, Bellevue, WA) to measure changes in forearm volume in response to inflation of venous congesting cuffs. Drug effects were expressed as the ratio of blood flow in the intervention to the control arm (21,22), where predose ratio was defined as 100%. Wrist cuffs were inflated to suprasystolic pressures defined as 100% (20,21). "Residual renal clearance was estimated taking the arithmetic mean of renal urea clearance and renal creatinine clearance (to correct for tubular creatinine secretion) and is normalized to 1.73 m² body surface area.

per dose level; Clinalfa). In a previous experiment, acetylcholine-dependent vasodilation was assessed on three different days at the same time of day in intervals of least 1 wk in eight healthy humans. The day-to-day coefficient of variation for repeated forearm blood flow measurements to acetylcholine was assessed as suggested by Petrie et al. (23) and was between 7% and 20%. Only forearm blood flow during the highest dose of the substances infused was used for correlation analysis to evaluate stimulation and inhibition of NO bioactivity. To be consistent with the established method at our institution, we also infused lower doses. Thus drug accumulation has to be acknowledged.

Table 1. Clinical parameters, l-arginine, ADMA, SDMA, and the l-arginine/ADMA ratio in patients on chronic peritoneal dialysis (n = 37)

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<th>Male/female</th>
<th>Age (yr)</th>
<th>Peritoneal dialysis since (mo)</th>
<th>Patients with coronary heart disease (n)</th>
<th>Patients with cerebrovascular disease (n)</th>
<th>Patients with hypertension (n)</th>
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Values are presented as means ± SEM.

*Residual renal clearance was estimated taking the arithmetic mean of renal urea clearance and renal creatinine clearance (to correct for tubular creatinine secretion) and is normalized to 1.73 m² body surface area.

ADMA indicates asymmetrical dimethyl l-arginine; SDMA, symmetrical dimethylarginine.

Analytical Methods

Blood lipids and urine parameters were determined by standard laboratory methods. For determination of l-arginine, ADMA, and its biologically inactive isomer symmetrical dimethylarginine (SDMA), venous blood was taken and plasma separated and stored at −30°C until batch analysis. Analysis was performed by HPLC as described previously (24). The coefficients of variation determined by dividing the SD by the mean from repeated analysis of a pooled plasma sample (n = 10) for interassay and intra-assay variations were <3% for all analytes. The detection limit for arginines was 0.04 μmol/L. l-arginine, ADMA, and SDMA plasma concentrations in a cohort of healthy subjects (n = 40; 18 women, 22 men; age: 54 ± 2 yr) were 80 ± 3 μmol/L,
0.47 ± 0.02 μmol/L, and 0.46 ± 0.01 μmol/L assessed with this method, respectively (unpublished historical data).

**Statistical Analyses**

Outcome parameters were tested for normal distribution using the Kolmogorov–Smirnov test and log-transformed if not normally distributed. The dose-response curves for vasoactive substances were analyzed by Friedman ANOVA. Correlations were calculated using Pearson correlation and only forearm blood flow responses during the highest dose of the vasoactive substances were analyzed. Statistica software version 6.0 (StatSoft, Tulsa, OK) was used for all analyses. P < 0.05 was considered the level of significance. Data are presented as means ± SEM.

**Results**

Clinical parameters and plasma l-arginine, ADMA, SDMA, and the l-arginine/ADMA ratio are presented in Table 1. Mean baseline forearm blood flow was 3.4 ± 0.2 ml/min per 100 ml tissue. Infusion of acetylcholine (Figure 1a) and nitroglycerine (Figure 1b) into the brachial artery caused a dose-dependent increase of blood flow (both P < 0.001, ANOVA) and l-NMMA (Figure 1c) decreased the blood flow in the infused forearm (P < 0.001; ANOVA).

Baseline forearm blood flow correlated negatively with plasma ADMA (P < 0.05) (Figure 2a) but had no association with plasma l-arginine, SDMA, or the l-arginine/ADMA ratio. A strong association between percent changes of forearm blood flow ratio over baseline in response to l-NMMA (4 μmol/min) with ADMA (P < 0.0005) (Figure 2b) could be observed. No correlation between l-NMMA (4 μmol/min) reactivity and l-arginine, SDMA or the l-arginine/ADMA ratio was detectable. Percent changes of forearm blood flow ratio over baseline in response to acetylcholine (100 nmol/min) tended to correlate positively with l-arginine (P = 0.05) and the l-arginine/ADMA ratio (P = 0.06). No association between acetylcholine (100 nmol/min) and ADMA (Figure 2c) or SDMA could be observed. Percent changes of forearm blood flow ratio in response to nitroglycerine (16 mmol/min) did not correlate with any parameter (Table 2).

**Discussion**

This study demonstrates that ADMA is strongly associated with basal NO bioactivity as assessed by the effects of intra-arterial l-NMMA on forearm blood flow. ADMA is not related to acetylcholine- or nitroglycerine-stimulated vasodilation in renal failure patients.

The NO synthase inhibitor l-NMMA reduces basal endothelial NO formation (25). Our data suggest that the l-NMMA–induced reduction in forearm blood flow ratio is influenced by ADMA, presumably by an interaction with NO production *in vivo*. Elevated ADMA concentrations were shown to induce microvascular lesions in animals (26). Reduced l-NMMA-induced vasoconstriction in PD patients with high ADMA could be partly caused by structural alterations of the vessels, *i.e.*, microvascular muscular hypertrophy or fibrosis. The inverse correlation of ADMA with baseline forearm blood flow is in accordance with results demonstrating that ADMA substantially increases vascular resistance in healthy humans (9). Basal vascular tone is to a large extent regulated by NO (27), which supports the assumption that elevated ADMA may influence constitutive NO formation also in chronic renal failure (28).
Baseline forearm blood flow found in PD patients was lower and the \(l\)-NMMA response was smaller than previously measured in healthy subjects at our institution (21). Plasma ADMA concentrations found in our study cohort were higher than values reported for healthy subjects from our laboratory (24) (unpublished data for older subjects) and others (29). This supports the notion that elevated ADMA might influence endothelial NO release in PD patients.

Circulating ADMA has been assumed to affect shear stress–activated vasodilation of the brachial artery (10–13). Increasing the \(l\)-arginine/ADMA ratio did not improve acetylcholine-induced vasodilation (30), whereas hemodialysis but not PD reduced ADMA concentrations and improved endothelium-dependent vasodilation measured by FMD in renal failure patients (31). Interestingly, no association between ADMA and acetylcholine-induced vasodilation could be observed in our cohort. However, results from FMD measurements are not comparable to receptor-stimulated vasodilation of arterial resistance vessels (18,19). This discrepancy may be caused by a different relative contribution of NO to the vasodilatory process (18). Nevertheless, reduced acetylcholine-stimulated forearm vasodilation as well as impaired FMD have been demonstrated in some studies to be both predictive of cardiovascular events in patients with coronary artery disease (32,33) and hypertension (34,35). Vallance et al. demonstrated that exogenously administered ADMA reduces endothelium-dependent vasodilation in healthy volunteers (4). Local administration of ADMA yields a much higher increase of ADMA concentrations than seen in this study. Thus, this experimental setting is at variance with the comparatively small intersubject differences in PD patients. It is unknown if a substantial increase in ADMA plasma concentrations also impairs acetylcholine-induced vasodilation in PD patients. The finding that ADMA is not related to acetylcholine-induced vasodilation in our cohort may be caused by the fact that muscarinic receptor stimulation cannot be completely abolished by infusion of \(l\)-NMMA (36,37), indicating that other factors than NO alone could contribute to the observed vasodilation. Furthermore, reduced stimulated NO bioavailability found in renal failure patients (2) could be secondary to diminished cofactors of NO synthesis such as tetrahydrobiopterin (38) or increased breakdown of NO, e.g., by reactive oxygen species, which were not measured in this study. Parasympathetic function is compromised in patients with ESRD (39). Parasympathetic neuropathy may be associated with reduced acetylcholine receptor numbers (40), which might confound acetylcholine responsiveness. The endothelium-derived hyperpolarization factor contributes to a large extent to acetylcholine-mediated vasodilation of isolated veins from dialysis patients (41) and is not influenced by NO synthase inhibition (42). In addition, endothelin is elevated in PD patients (43) and may influence vascular tone or function in renal failure patients (44). Furthermore, measurement of forearm blood flow is subject to considerable day-to-day variability. The coefficient of variation for three measurements at intervals of at least 1 wk was between 7% and 20% for different doses of acetylcholine at our institution (unpublished observation), which is in the same range as reported in other studies (23). These factors might contribute to the lack of association between acetylcholine-induced vasodilation and ADMA.

ADMA is predictive for the overall mortality and cardiovascular outcome in renal failure patients (5) but does not correlate with acetylcholine-induced vasodilation in our study. Although endothelial dysfunction is a risk factor in several car-

Figure 2. Scatterplot of ADMA concentrations versus absolute baseline forearm blood flow (a) and changes in forearm blood flow ratio over baseline during intra-arterial \(l\)-NMMA (4 \(\mu\)mol/min) (b) and acetylcholine (100 nmol/min) (c).
diovascular diseases, e.g., heart failure (45), its prognostic signif-
icance in uremic patients is at least unclear. Basal NO formation as assessed by l-NMMA–induced vasoconstriction was inversely associated with ADMA in our cohort of renal failure patients. Reduced endothelial synthesis of the antiatherogenic molecule NO might increase the risk for cardiovascular events by other factors than impaired endothelium-dependent vasodilation alone.

Plasma concentrations of the substrate for NO synthesis, l-arginine (46), tended to correlate with acetylcholine-stimulated vasodilation, which might indicate some relationship between increased substrate availability and improved stimulated NO production. However, even a substantial pharmacologic increase in l-arginine levels has little impact on endothelium-dependent vasodilation in patients with chronic renal failure (30). This could be caused by adequate substrate availability in these patients. However, it has been reported that prolonged ADMA exposure is required for steady-state effects in healthy volunteers (47). ADMA and l-arginine have to be transported into endothelial cells to act on intracellular NO synthesis. Thus, the effects of acute l-arginine administration and long-term elevated l-arginine concentrations may not be comparable.

In this study, ADMA was lower than reported from patients on hemodialysis (48), which is consistent with reports describing lower ADMA in PD patients compared with hemodialysis patients (31,49). ADMA diffusion into peritoneal dialysis solutions obviously enables an enhanced clearance of ADMA. However, plasma concentrations of ADMA found in similar populations differ considerably subject to laboratory methods used. Mean ADMA plasma levels of 2.1 μmol/L were described in PD patients in a previous report (49), which is much higher than in this study. This difference is a result of methodological differences between laboratories. This lack of analytical standardization complicates comparisons of absolute ADMA values between studies.

In conclusion, ADMA is strongly associated with basal NO release in resistance arteries of PD patients in vivo. No relationship between ADMA and acetylcholine-stimulated endothelium-dependent vasodilation could be observed. This might be of particular interest for future studies evaluating the interrelations between ADMA, endothelial function, and cardiovascular risk.

References
11. Savvidou MD, Hingorani AD, Tsikas D, Fröhlich JC, Val-

Table 2. Linear associations of l-arginine, ADMA, SDMA, and the l-arginine/ADMA ratio with basal FBF and percent changes in FBF ratio over baseline during infusion of acetylcholine (100 nmol/min), nitroglycerine (16 mmol/min), and l-NMMA (4 μmol/min)

<table>
<thead>
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<th>l-arginine</th>
<th>ADMA</th>
<th>SDMA</th>
<th>l-arginine/ADMA</th>
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<td>r</td>
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<td>FBF</td>
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<td>Acetylcholine</td>
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<td>Nitroglycerine</td>
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<td>l-NMMA</td>
<td>0.27</td>
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FBF indicates forearm blood flow.


39. Zoccali C, Ciccarelli M, Maggiore Q: Defective reflex con-


