Reduced Agonist-Induced Endothelium-Dependent Vasodilation in Uremia Is Attributable to an Impairment of Vascular Nitric Oxide

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Current concepts for the explanation of endothelial dysfunction and accelerated atherosclerosis in uremia propose a reduced vascular bioavailability of nitric oxide (NO). The aim of the present study was to test the contributions of NO and NO/prostacyclin (PGI2)-independent mechanisms to both baseline vascular tone and agonist-induced endothelium-dependent vasodilation in patients on hemodialysis (HD). In 10 HD patients and eight matched healthy control subjects, forearm blood flow (FBF) was measured at rest and during intrabrachial infusions of norepinephrine (NE; endothelium-independent vasoconstrictor, 60, 120, and 240 pmol/min) and N-monomethyl-L-arginine (blocker of NO synthases, 16 μmol/min). After inhibition of cyclo-oxygenase by ibuprofen (1200 mg orally), endothelium-dependent and -independent vasodilation was assessed by infusion of acetylcholine (ACh; 1, 5, 10, 50, 100, and 300 nmol/min) and sodium-nitroprusside (2.5, 5, and 10 µg/min). NO/PGI2-independent vasodilation was tested by equal infusions of ACh during NO clamp. N-monomethyl-L-arginine reduced resting FBF to a comparable degree in both groups. Vascular responses to ACh were reduced in HD (P = 0.003 versus control by ANOVA), whereas those to sodium nitroprusside were mainly at control level. Infusion of ACh during NO clamp caused a similar increment of FBF in both groups. NO-mediated vasodilation as calculated by the difference between ACh-induced responses without and with NO clamp was substantially impaired in HD (P < 0.001) compared with control. In HD patients, baseline NO-mediated arteriolar tone is at control level. This study provides first evidence that endothelial dysfunction of uremic patients as shown by reduced agonist-induced endothelium-dependent vasodilation is attributable to reduced stimulation of NO, whereas the NO/PGI2-resistant portion of ACh-mediated vasodilation is unaffected.


Uremia is a state of excessive cardiovascular mortality (1). In addition, renal insufficiency is considered to be an adverse prognostic factor for underlying cardiovascular disease (2). The mechanisms by which uremia promotes cardiovascular complications are poorly understood. Apart from conventional risk factors, it has been shown that plasma levels of asymmetric dimethyl-arginine (3), homocysteine (4), and inflammatory mediators (5–7) are positively correlated to mortality in uremia. Moreover, there is convincing evidence that end-stage renal disease (ESRD) is a state of increased oxidative stress (8,9). The various potential explanations for accelerated vascular disease in uremia share one similarity: They propose a generally reduced bioavailability of endothelium-derived nitric oxide (NO) caused either by inhibition of NO synthases (NOS) or by increased degradation of NO itself. A critical review of the literature, however, unveils serious discrepancies in this context. Systemic NO synthesis in uremia was shown to be increased (10–14) as well as reduced (15,16) dependent on the individuals studied and on the experimental setup used. Moreover, an impairment of stimulated vascular NO was suggested to explain the compromised agonist-induced, endothelium-dependent vasodilation observed in animal models of chronic renal failure (17,18). The latter assumption, however, has never been tested in humans. A significant portion of agonist-induced endothelium-dependent vasodilation is resistant to concomitant blockade of NOS and cyclo-oxygenases and has been attributed to the release of endothelium-dependent hyperpolarizing factors (EDHF, reviewed in [19]). It is conceivable that uremia interferes with EDHF-associated signaling pathways, too. Such results have been obtained in isolated vessels of 5/6 nephrectomized rats (20). As agonist-induced, endothelium-dependent vasodilation has prognostic significance (21,22), it would be desirable to know which portion of the complex vascular response to agonists is impaired. This question is important for our understanding of uremic endothelial dysfunction as well as for future therapeutic interventions. We therefore asked the following questions: (1) How much does NO contribute to baseline vascular tone in uremia compared with control? (2) What are the contributions of NO and NO/prostacyclin (PGI2)-independent...
mediators to endothelium-dependent vasodilation in uremia? (3) Which portion(s) of the agonist-induced vascular response is(are) compromised compared with control? To answer these questions, we performed invasive vascular testing in patients who were on hemodialysis (HD) and in healthy control subjects by using the forearm blood flow (FBF) technique.

Materials and Methods

The study protocol was approved by the University of Dresden ethics committee. Ten stable, nondiabetic male patients who were on regular HD and eight healthy volunteers matched for age, gender, height, weight, and smoking habits were recruited, and written informed consent was obtained before any investigation was started. All HD patients enrolled were on a transplant waiting list. Physical examination, electrocardiogram, exercise testing, and laboratory screening were performed regularly in this context and revealed no evidence for clinically relevant atherosclerotic disease. Five HD patients had mild hypertension that required drug treatment (angiotensin-converting enzyme inhibitors, n = 4; β-blockers, n = 3). Antihypertensive medication was withdrawn 48 h before the beginning of the study. HD patients were studied in the morning during their short dialysis-free interval. Their actual weight did not exceed the estimated dry weight by >2 kg. In healthy control subjects, hypertension or underlying vascular disease was ruled out by physical examination. Cigarettes, alcohol, and all caffeine-containing beverages were withheld for 12 h before the study. All investigations were performed in a quiet room kept at a constant temperature of between 22 and 24°C. Each participant was supine, with both forearms resting slightly above heart level.

Measurement of FBF

FBF was measured simultaneously in both arms by venous occlusion plethysmography as described previously (23). In HD patients, only the arm that did not bear the arteriovenous fistula was available for FBF recordings. Pressure of the constricting cuffs of both upper arms was set at 40 mmHg. Mercury-in-silastic strain gauges were wrapped around the widest parts of the forearms and connected to a calibrated venous occlusion plethysmogaph (Gutmann Medizinelektronik, Eursburg, Germany). The brachial artery of the nondominant arm (or the arm without arteriovenous fistula, respectively) was cannulated for drug infusion. We considered the possibility that repeated puncture of the brachial artery might be related to arterial trauma. However, we used a very thin (27 G) steel needle (Coopers Needle Work, Birmingham, UK), which was inserted under ultrasound guidance by a single experienced investigator (I.P.). We have analyzed our previous experience in doing >500 such cannulations—most of which have been done repeatedly—and found no evidence for arterial changes by ultrasound examination. Discussion of the subject with our local ethical committee resulted in the consensus that our approach is ethically acceptable for all involved.

After cannulation of the brachial artery, saline was infused for 20 min to establish baseline conditions in each protocol. Individual measurements of FBF lasting 10 s were made every 15 s for 2.5 min during each dose of agent administered. The blood flow of the hands was excluded by a wrist cuff inflated to a suprasystolic pressure (220 mmHg) during each measurement period. BP was measured at baseline and at the end of each infusion period. During each protocol, the infusion rate was kept constant at 1 ml/min. At the end of each FBF measurement, BP was determined at the left A. dorsalis pedis.

Experimental Protocols

All participants underwent three experimental sessions. Between individual sessions, a period of at least 7 d was given.

Influence of N-Monomethyl-L-Arginine on Baseline FBF. N-monomethyl-L-arginine (L-NMMA), an inhibitor of NOS, was infused at 16 µmol/min for 10 min, and FBF measurements were performed. This dose of L-NMMA has been shown to mediate maximal inhibition of both baseline and stimulated vascular NO synthesis in the forearm (24).

Acetylcholine-Induced Endothelium-Dependent Vasodilation in Either the Absence or the Presence of L-NMMA (NO Clamp). One hour before this protocol, participants received 1200 mg of ibuprofen orally. This dose has been used previously for blockade of endothelial PGI2 generation (25).

Evaluation of Endothelium-Independent Vasodilation and Constriction. After determination of baseline FBF, norepinephrine (NE) was infused into the brachial artery at three increasing doses (60, 120, and 240 µmol/min). FBF measurements were performed at the end of each 5-min dosing period. After 30 min of rest, participants received increasing doses of SNP (2.5, 5, and 10 µg/min). Again, FBF measurements were performed at the end of each dosing period (5 min).

Drugs

Ibuprofen (Jenaprofen) was obtained from Jenapharm (Jena, Germany), L-NMMA was from Clinalfa (La¨ufelfingen, Switzerland), ACh (Miochol E) was obtained from Ciba Vision (Germering, Germany), SNP (nipruss) was from Schwarz Pharma (Monheim, Germany), and NE (Arterenol) was from Aventis (Frankfurt, Germany). All agents used were dissolved in physiologic saline except for SNP, which was dissolved in glucose 5% avoiding exposure to light.

Statistical Analyses

One FBF determination consisted of 10 single FBF measurements. The final five blood flow recordings for each infusion step were used to calculate mean FBF. Absolute values of FBF are expressed in milliliters per deciliter of forearm tissue per minute. Infusion effects of vasodilators (ACh and SNP) are given as difference between the absolute FBF during drug infusion and the FBF measured before drug infusion (ΔFBF = [FBF observed] − [baseline FBF]) to account for the slightly higher baseline FBF values in the HD group. If the percentage increase had been reported instead, then the results in the group with the higher baseline level would have been underestimated and the difference between groups would have been overemphasized. Accordingly, infu-sion effects of vasoconstrictors (L-NMMA and NE) are shown as percentage change from baseline FBF ([FBF observed/baseline FBF × 100] − 100), with baseline defined as zero. Results are presented as mean ± SEM. Comparison of group characteristics and vascular responses to L-NMMA at rest were performed using t test. Dose-response curves to ACh and SNP were analyzed by two-way ANOVA for repeated measurements. Values of P < 0.05 were considered statistically significant.

Results

Arterial puncture was performed without complications in all participants. Substances were tolerated well by all partici-
pants. BP and heart rate remained stable in each individual during all experimental sessions (data not shown). In healthy volunteers, FBF in the control arm was constant during each infusion protocol. Baseline characteristics of the participants in each group are given in Table 1.

<table>
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<th>Characteristic</th>
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<th>Control (n = 8)</th>
<th>P Value</th>
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<td>Age (yr)</td>
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<td>Height (cm)</td>
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<td>Weight (kg)</td>
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<td>Systolic BP (mmHg)</td>
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<td>67 ± 2</td>
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<td>Mean BP (mmHg)</td>
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Table 1. Baseline characteristics of HD patients and healthy control subjects

*HD, hemodialysis.

Influence of L-NMMA on Baseline FBF

Infusion of L-NMMA decreased FBF from 3.5 ± 0.7 ml/dl per min to 1.9 ± 0.3 by 43 ± 4% in HD and from 2.4 ± 0.4 ml/dl per min to 1.2 ± 0.2 by 47 ± 7% in control. Comparison of the infusion effects by t test revealed no difference between both groups (Figure 1A).

ACh-Induced Endothelium-Dependent Vasodilation in Either the Absence or the Presence of L-NMMA (NO Clamp)

Graded infusions of ACh increased FBF from 3.6 ± 0.6 ml/dl per min to 16.9 ± 1.8 in HD and from 1.8 ± 0.2 to 22.2 ± 1.4 in control subjects. Analysis of the infusion effects (ΔFBF) by ANOVA for repeated measurements revealed a significant difference between HD and control (P = 0.003; Figure 2A).

After establishment of the NO clamp, FBF in each group was comparable to baseline FBF before infusion of ACh in the first part of the protocol (3.0 ± 0.5 ml/dl per min in HD and 1.8 ± 0.2 in control). During NO clamp, infusions of ACh increased FBF to 15.9 ± 1.8 in HD and to 13.5 ± 1.7 in control. Comparison of the infusion effects of ACh (ΔFBF) by ANOVA for repeated measurements showed no difference between groups. Comparison of infusion effects of ACh dose-by-dose (t test) during NO clamp revealed a significantly increased response at 100 nmol/min in HD compared with control (P < 0.05; Figure 3A).

To visualize ACh-induced, NO-mediated vasodilation, we calculated the difference between ACh-induced responses without NO clamp and ACh-induced responses during NO clamp. It was substantially impaired in HD (P < 0.001 by ANOVA) compared with control (Figure 3B). In Figure 4, the contribution of NO-dependent and NO/PGL2-independent relaxation is given in percentage of total vasodilation elicited by each dose of ACh in each group.

Evaluation of Endothelium-Independent Vasodilation and Constriction

Infusions of NE dose-dependently decreased FBF from 2.6 ± 0.3 ml/dl per min to 1.4 ± 0.2 by 40 ± 3% in HD and from 2.5 ± 0.3 to 1.6 ± 0.2 by 45 ± 3% in control. Comparison of the infusion effects (percentage change of baseline FBF) by ANOVA for repeated measurements showed no difference between groups (Figure 1B).

Infusions of SNP dose-dependently increased FBF in both groups: From 2.7 ± 0.4 ml/dl per min to 13.6 ± 1.8 in HD and from 2.9 ± 0.3 ml/dl per min to 16.7 ± 1.3 in control. Although the infusion effect of SNP as measured by ΔFBF tended to be reduced in HD, statistical analysis by ANOVA for repeated measurements revealed no difference between groups (Figure 2B). To rule out a general stiffening of forearm resistance vessels in HD patients, we investigated peak FBF after 5 min of forearm ischemia in all participants of this study in a separate session. The results demonstrate comparable vascular responses in both groups (HD, from 2.9 ± 0.5 to 17.0 ± 1.5; control, from 2.5 ± 0.6 to 16.8 ± 1.3 ml/dl per minute; P = 0.88 by ANOVA).

Discussion

This study was conducted to test the contribution of NO to both baseline and agonist-stimulated arteriolar tone in uremic patients by using the FBF technique. This method has the advantage to test vascular responses in intact vessels within the individual physiologic milieu (26). Undoubtedly, bilateral plethysmography is the preferable method for testing the influence of drugs and mediators on FBF (26,27). However, all HD patients enrolled in our studies had patent arteriovenous fistulae on one arm, which makes bilateral plethysmography impossible. In the control group, we used the two-arm approach. Comparison of bilateral to unilateral plethysmography within this group revealed no significant differences between both methods (data not shown). In addition, considering the constancy of BP and heart rate throughout the experiments, it is unlikely that systemic effects of the drugs infused have biased our results.

We found that in HD patients without signs of clinically relevant atherosclerosis, NO contributes equally to baseline
vascular tone as in control subjects. In a previous study, we demonstrated comparable results (23). In the present study, we repeated our observations by using an improved experimental setup: First, we gave a considerably higher dose of L-NMMA, which was shown recently to mediate complete inhibition of forearm vascular NO synthesis (24). Second, we infused L-NMMA in a separate experimental session. In this way, we could completely rule out sustained effects of preceding drug infusions. Again, our results do not support the hypothesis that uremia is a state of NO impairment under baseline conditions in general.

Furthermore, we tested endothelium-dependent vasodilation in HD patients and control subjects by graded infusions of ACh at six doses between 1 and 300 nmol/min. In comparable studies, measurements of endothelium-dependent vasodilation are usually confined to the upper dose range of ACh (approximately between 50 and 300 nmol/min). We started infusions of ACh at markedly lower doses because it has been demonstrated that vasodilation induced by the agonist in that dose range is primarily dependent on NO (24). We made the observation that the impairment of endothelium-dependent vasodilation in uremic individuals is most significant at higher doses of ACh (50 nmol/min or more), whereas relaxations induced by low-dose ACh largely compare with those of healthy control subjects. These findings were substantiated further by infusions of ACh during NO clamp. The latter technique has been used successfully to quantify the involvement of NO in the vasomotor properties of various compounds (28–31). In healthy control subjects, NO significantly contributed to ACh-induced vasodi-
lation ranging from a portion of approximately 90% at the lowest dose to 40% at the highest dose of ACh. Vice versa, with increasing doses of ACh, the portion of NO/PGL2-independent vasodilation rose continuously from approximately 10 to 60% (Figure 4). These results are in good agreement with those reported by Dawes et al. (24). In HD patients, we observed a different pattern of vascular response to increasing doses of ACh: Vasodilations induced by low-dose ACh (up to 5 nmol/min) were almost completely inhibited by L-NMMA. In the higher dose range of ACh (50 nmol/min and above), we observed a sharp decrease of sensitivity to L-NMMA, indicating that in this situation, agonist-induced vasodilation almost exclusively depends on NO/PGL2-independent mechanisms. These results provide first evidence that uremic endothelial dysfunction as measured by agonist-induced endothelium-dependent vasodilation results mainly from an impairment of its NO component. Our experimental design does not allow direct insight into the mechanisms of NO impairment. In the literature, the presence of endogenous competitive NOS inhibitors in uremia is frequently used to explain this phenomenon (32). Generally, competitive inhibition of an enzyme results in a rightward shift of the dose-response curve of an agonist. Our results, however, are not compatible with that particular pattern of response as vascular release of NO at low dose ACh was unaffected in uremia. It therefore is unlikely that such compounds are mainly responsible for the NO impairment observed. Comparable conclusions were drawn by Morris et al. (33) on the basis of their organ bath experiments investigating the vasodilatory properties of small subcutaneous arteries of uremic individuals. A more likely explanation for the characteristics of NO stimulation observed in our studies could be uncoupling of endothelial NOS (eNOS), which in turn may be caused by substrate and/or co-factor deficiency occurring at a certain degree of agonist stimulation. In this context, uremia-associated changes in L-arginine transport (responsible for the delivery of substrate to NOS [34]) as well as alterations in pteridine-metabolism (providing tetrahydrobiopterin as a critical co-factor of NO synthesis [35]) have been observed. Uncoupling of eNOS not only results in reduced NO production but also is associated with increased oxidative stress by generation of superoxide anions, which in turn cause further NO degradation by forming peroxynitrite. The presence of high levels of oxidative stress in uremia has been demonstrated in various studies (8,36,37).

Apart from eNOS itself, there may be other factors potentially involved in the generation of uremic oxidative stress,
including NAD(P)H oxidase (38), hyperhomocysteinemia (39), and myeloperoxidase (40). The potential contribution of these mechanisms to the observed impairment of vascular NO has to be clarified in further studies.

In contrast, the NO/PGL2-independent portion of ACh-induced vasodilation was not reduced in HD patients compared with control subjects. It was even increased in the higher dose range of ACh, where NO-mediated vasodilation was substantially impaired. This is compatible with previous observations proposing an interaction between NO- and EDHF-mediated vasodilation in HD patients was beyond the scope of the present study but will be subject to further investigation.

In summary, in HD patients without overt atherosclerotic disease, we found no evidence for vascular NO deficiency at rest. By using the NO clamp technique, we could demonstrate that endothelial dysfunction in uremic individuals as measured by agonist-induced endothelium-dependent vasodilation is attributable to an impairment of its NO component, whereas NO/PGL2-independent vasodilation is unaffected or even in-tributable to an impairment of its NO component, whereas by agonist-induced endothelium-dependent vasodilation is at-
tent vasodilation in HD patients was beyond the scope of the present study but will be subject to further investigation.

and myeloperoxidase (40). The potential contribution of these including NAD(P)H oxidase (38), hyperhomocysteinemia (39), and myeloperoxidase (40). The potential contribution of these mechanisms to the observed impairment of vascular NO has to be clarified in further studies.

References


