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Nitric oxide in chronic renal failure

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Nitric oxide in chronic renal failure. Endothelium-derived nitric oxide (NO) is critically involved in the regulation of a wide variety of vascular functions. It had been hypothesized that a deficiency of vascular NO might be involved in the accelerated atherosclerosis and dramatic cardiovascular mortality observed in patients with chronic renal failure. At present it is difficult to measure authentic NO in vivo. An alternative is to study NO by its effect on vascular tone by using the forearm blood flow technique. In this way, studies demonstrated an unimpaired availability of NO under baseline conditions but a profound reduction of agonist-induced endothelium-dependent vasodilatation in uremic patients. Further investigation showed that the latter phenomenon is mainly attributable to a reduced availability of vascular NO upon agonist stimulation, while the NO-independent mechanism(s) appear(s) to be intact in this setting. Explanations for this finding include an uncoupling of NO synthase induced by cofactor deficiency, and/or a reduced NO availability caused by high levels of oxidative stress. Recent data suggest only a minor role for cytochrome-P450 2C9-dependent pathways in this context. Future studies have to show which mechanisms are most relevant, and whether they are sensitive to therapeutic intervention.

Since the discovery of the endothelium-derived relaxing factor and its subsequent identification as nitric oxide, there was hardly one molecule which has gained so much interest in medical science. While nitric oxide is involved in a wide variety of physiologic functions, its role as an endothelium-derived mediator has been most extensively studied in cardiovascular medicine. In this context nitric oxide has turned out to be a central mediator involved in various vascular functions. Because of its vasodilatory, antiproliferative, and antithrombogenic properties, nitric oxide has been predominantly regarded as an antiatherogenic compound. It is now clear, however, that under conditions of increased oxidative stress nitric oxide may be involved in vascular damage and atherosclerosis, too [1]. Alterations of the vascular nitric oxide system have been observed very early in the development of atherosclerosis. Such early stage of atherosclerosis, termed endothelial dysfunction, is associated with a reduced bioavailability of nitric oxide and can be assessed by an impairment of endothelium-dependent vasodilatation [2].

Uremia is a state of increased cardiovascular morbidity and mortality and therefore believed to be associated with accelerated atherosclerosis [3, 4]. It is therefore not surprising that considerable scientific interest focused on alterations of the vascular nitric oxide system in uremic subjects to find explanations and therapeutic options for their excessive cardiovascular mortality. The discovery of endogenous nitric oxide synthase (NOS) inhibitors gave rise to the hypothesis that uremia might be a state of nitric oxide deficiency [5].

At present it is very difficult, if not impossible, to measure authentic nitric oxide in vivo. Therefore various attempts have been made to estimate total body nitric oxide production in animal models as well as in patients with chronic renal failure by a variety of surrogate parameters. Unfortunately, these studies have revealed controversial results. For example, by determination of arginine-to-citrulline conversion, Wever et al [6] found a reduced total nitric oxide production in patients with advanced renal insufficiency. In contrast, by using a comparable experimental setup, Lau et al [7] reported on enhanced nitric oxide synthesis in patients on hemodialysis. Measurements of plasma levels of stable nitric oxide metabolites or of concentrations of exhaled nitric oxide have resulted in discrepant findings, too [8, 9]. In fact the literature contains lines of evidence supporting enhanced as well as reduced nitric oxide synthesis in uremia. These controversies may be related to the fact that nitric oxide can be metabolized in different ways. Thus measurements of one stable metabolite do not necessarily reflect total nitric oxide synthesis. In addition, determinations of nitric oxide synthesis do not provide reliable information about its bioavailability. To aggravate this situation it is unclear to which extent total body nitric oxide measurements are representative for vascular nitric oxide production [10].

A promising way to circumvent these difficulties is to study the effects of NOS stimulation and subsequent nitric oxide release directly by measurement of the resulting changes in vascular tone. One such approach determines the dilatation of the brachial artery in response to postischemic increase in blood flow. In this way Kari et al [11] were the first to demonstrate an impairment of flow-mediated dilatation in children with renal insufficiency indicating that uremic intoxication per se is associated with endothelial dysfunction and impaired bioavailability of nitric oxide upon shear stress–induced stimulation of

the endothelium. Unfortunately, it is not possible to study the contribution of nitric oxide to baseline vascular tone by this method. Such more complex vascular testing can be accomplished by using the forearm blood flow technique. Here locally active doses of vasoactive substances are infused into the brachial artery of one arm and the resulting changes in forearm blood flow are measured by venous occlusion plethysmography. In this way we could repeatedly show that vasoconstriction of forearm resistance vessels in response to the NOS inhibitor L-NMMA is not reduced in otherwise healthy uremic patients [12, 13]. Together with the previous finding that nitric oxide generation in uremic platelets is even enhanced [14, 15] our results do not support the hypothesis of a general impairment of nitric oxide synthesis in renal insufficiency.

Further experiments in comparable groups of patients, however, demonstrated a profound impairment of vasodilatation upon endothelial stimulation with muscarinic agonists such as carbachol or acetylcholine [12, 16]. These findings confirmed the results obtained by Kari et al [11] and provided further evidence that uremia is a state of endothelial dysfunction. However, these results per se still cannot be regarded as evidence for an impairment of stimulated nitric oxide release in this setting as cyclooxygenase is stimulated by agonists, too, which in turn generates vasodilatory prostacyclin I_2 (PGI_2). Furthermore, a significant portion of agonist-induced endothelium dependent vasodilation is resistant to concomitant blockade of NOS and cyclooxygenases and has been attributed to the release of endothelium-dependent hyperpolarizing factors (EDHF) (reviewed in [17]). 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 [18]. We have therefore performed further experiments in uremic subjects by employing the nitric oxide clamp technique. The latter approach allowed us to study the contributions of nitric oxide and nitric oxide/PGI_2-independent mechanisms to agonist-induced vasodilatation separately. By using an extended dose range of acetylcholine (1 to 300 nmol/min) we could demonstrate a preserved nitric oxide stimulation in response to lower doses of this muscarinic agonist. Infusions of higher doses of acetylcholine, however, were associated with a brisk and substantial decline of nitric oxide–mediated vasodilatation in uremic subjects while those relaxations elicited by nitric oxide/PGI_2-independent mechanisms were at control level or even increased. This study provides first evidence in humans that uremic endothelial dysfunction as measured by agonist-induced, endothelium-dependent vasodilatation is attributable to an impairment of vascular nitric oxide. Furthermore, the particular pattern of vascular responses observed may help to understand the underlying mechanisms of nitric oxide impairment in uremia. Generally, the latter may result from decreased nitric oxide generation or increased nitric oxide breakdown by oxidative compounds. In the literature, decreased nitric oxide generation is usually explained by the presence of endogenous competitive NOS inhibitors [asymmetrical dimethylarginine (ADMA)] [19]. In fact, 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 nitric oxide at baseline and during low-dose acetylcholine was unaffected in uremia. It is therefore unlikely that constant levels of such compounds are mainly responsible for the nitric oxide impairment observed. This view is further substantiated by the fact that neither acute [20] nor chronic [21] supplementation with L-arginine was able to improve endothelial function in patients with renal failure. There is one report in the literature showing an increase of ADMA generation in endothelial cells in response to shear stress [22]. However, whether the magnitude of the observed changes can sufficiently explain such profound impairment of stimulated nitric oxide as seen in our study requires further investigation.

A more likely explanation for the characteristics of nitric oxide stimulation observed in our studies could be uncoupling of endothelial NOS (eNOS), which in turn may be caused by substrate and/or cofactor 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 [23]) as well as alterations in pteridine metabolism (providing tetrahydrobiopterin as a critical cofactor of nitric oxide synthesis [24], have been observed. Further studies are needed to clarify this issue.

Uncoupling of eNOS does not only result in reduced nitric oxide production but is also associated with increased oxidative stress by generation of superoxide anions which in turn cause further nitric oxide degradation by forming peroxynitrite. The presence of high levels of oxidative stress in uremia has been demonstrated in various studies [25–27].

Apart from eNOS itself, there may be other factors potentially involved in the generation of uremic oxidative stress, including NAD(P)H oxidase [28], hyperhomocysteinemia [29], and myeloperoxidase [30]. Recently, a cytochrome P450 (CYP 2C)-dependent epoxygenase has been shown to generate reactive oxygen species which interfere with the bioavailability of nitric oxide and consequently interfere with nitric oxide–mediated vasodilatation and the expression of redox-sensitive genes [31]. The latter mechanism appears to dominate in situations of endothelial dysfunction since a considerable improvement in the acetylcholine-induced nitric oxide–dependent vasodilatation of the forearm vasculature was recorded in patients with coronary heart disease when CYP 2C9 was inhibited [32]. We have recently tested whether this pathway contributes to uremic endothelial dysfunction, too. Inhibition of CYP 2C9 by sulfaphenazole was not

able to improve acetylcholine-induced, endothelium-dependent vasodilatation in hemodialysis patients without clinical signs of atherosclerotic disease [33]. This finding may indicate that vascular expression of CYP 2C9 and subsequent inactivation of nitric oxide by reactive oxygen species is not involved in early endothelial dysfunction of uremic subjects.

CONCLUSION

We have so far demonstrated that in a local vascular bed of uremic subjects the contribution of nitric oxide to baseline vascular tone is at control level. We provided first evidence in humans that uremic endothelial dysfunction as measured by agonist-induced endothelium-dependent vasodilatation is attributable to an impairment of nitric oxide. In this context uncoupling of eNOS and/or nitric oxide consumption by uremic oxidative stress are possible explanations for this finding while the role of ADMA remains controversial. Furthermore, CYP 2C9-derived reactive oxygen species are unlikely to be involved in early endothelial dysfunction of uremic subjects.

Taken together there are various mechanisms by which uremic intoxication might interfere with the bioavailability of vascular nitric oxide. Future studies have to show which of these mechanisms are most relevant and whether they are sensitive to therapeutic intervention.

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REFERENCES

1. Wever RM, Luscher TF, Cosentino F, Rabelink TJ: Atherosclerosis and the two faces of endothelial nitric oxide synthase. *Circulation* 97:108–112, 1998
2. Ross R: Atherosclerosis—An inflammatory disease. *N Engl J Med* 340:115–126, 1999
3. Lindner A, Charra B, Sherrard DJ, Scribner BH: Accelerated atherosclerosis in prolonged maintenance hemodialysis. *N Engl J Med* 290:697–701, 1974
4. Foley RN, Parfrey PS, Sarnak MJ: Epidemiology of cardiovascular disease in chronic renal disease. *J Am Soc Nephrol* 9:S16–S23, 1998
5. Ritz E, Vallance P, Nowicki M: The effect of malnutrition on cardiovascular mortality in dialysis patients: Is L-arginine the answer? *Nephrol Dial Transplant* 9:129–130, 1994
6. Wever R, Boer P, Hijmering M, *et al*: Nitric oxide production is reduced in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol* 19:1168–1172, 1999
7. Lau T, Owen W, Yu YM, *et al*: Arginine, citrulline, and nitric oxide metabolism in end-stage renal disease patients. *J Clin Invest* 105:1217–1225, 2000
8. Schmidt RJ, Baylis C: Total nitric oxide production is low in patients with chronic renal disease. *Kidney Int* 58:1261–1266, 2000
9. Matsumoto A, Hirata Y, Kakoki M, *et al*: Increased excretion of nitric oxide in exhaled air of patients with chronic renal failure. *Clin Sci (Colch)* 96:67–74, 1999
10. Baylis C, Vallance P: Measurement of nitrite and nitrate levels in plasma and urine—What does this measure tell us about the activity of the endogenous nitric oxide system? *Curr Opin Nephrol Hypertens* 7:59–62, 1998
11. Kari JA, Donald AE, Vallance DT, *et al*: Physiology and biochemistry of endothelial function in children with chronic renal failure. *Kidney Int* 52:468–472, 1997
12. Passauer J, Bussemaker E, Range U, *et al*: Evidence In vivo showing increase of baseline nitric oxide generation and impairment of endothelium-dependent vasodilation in normotensive patients on chronic hemodialysis. *J Am Soc Nephrol* 11:1726–1734, 2000
13. Passauer J, Pistrosch F, Bussemaker E, *et al*: Reduced agonist-induced endothelium- dependent vasodilatation in uremia is attributable to an impairment of vascular nitric oxide. *J Am Soc Nephrol* (in press)
14. Noris M, Remuzzi G: Uremic bleeding: Closing the circle after 30 years of controversies? *Blood* 94:2569–2574, 1999
15. Brunini TM, Yaqoob MM, Novaes Malagris LE, *et al*: Increased nitric oxide synthesis in uraemic platelets is dependent on L-arginine transport via system y(+)L. *Pflugers Arch* 445:547–550, 2003
16. Morris ST, McMurray JJ, Rodger RS, *et al*: Impaired endothelium-dependent vasodilatation in uremia. *Nephrol Dial Transplant* 15:1194–1200, 2000
17. Busse R, Edwards G, Feletou M, *et al*: EDHF: Bringing the concepts together. *Trends Pharmacol Sci* 23:374–380, 2002
18. Kalliovalkama J, Jolma P, Tolvanen JP, *et al*: Potassium channel-mediated vasorelaxation is impaired in experimental renal failure. *Am J Physiol* 277:H1622–H1629, 1999
19. Vallance P, Leone A, Calver A, *et al*: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339:572–575, 1992
20. Cross JM, Donald AE, Kharbanda R, *et al*: Acute administration of L-arginine does not improve arterial endothelial function in chronic renal failure. *Kidney Int* 60:2318–2323, 2001
21. Bennett-Richards KJ, Kattenhorn M, Donald AE, *et al*: Oral L-arginine does not improve endothelial dysfunction in children with chronic renal failure. *Kidney Int* 62:1372–1378, 2002
22. Osanai T, Saitoh M, Sasaki S, *et al*: Effect of shear stress on asymmetric dimethylarginine release from vascular endothelial cells. *Hypertension* 42:985–990, 2003
23. Mendes Ribeiro AC, Brunini TM, Ellory JC, Mann GE: Abnormalities in L-arginine transport and nitric oxide biosynthesis in chronic renal and heart failure. *Cardiovasc Res* 49:697–712, 2001
24. Yokoyama K, Tajima M, Yoshida H, *et al*: Plasma pteridine concentrations in patients with chronic renal failure. *Nephrol Dial Transplant* 17:1032–1036, 2002
25. Itabe H, Yamamoto H, Imanaka T, *et al*: Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. *J Lipid Res* 37:45–53, 1996
26. Ferraro B, Galli F, Frei B, *et al*: Peroxynitrite-induced oxidation of plasma lipids is enhanced in stable hemodialysis patients. *Kidney Int* 63:2207–2213, 2003
27. Cross JM, Donald AE, Nuttall SL, *et al*: Vitamin C improves resistance but not conduit artery endothelial function in patients with chronic renal failure. *Kidney Int* 63:1433–1442, 2003
28. Vaziri ND, Dicus M, Ho ND, *et al*: Oxidative stress and dysregulation of superoxide dismutase and NADPH oxidase in renal insufficiency. *Kidney Int* 63:179–185, 2003
29. Zhang X, Li H, Jin H, *et al*: Effects of homocysteine on endothelial nitric oxide production. *Am J Physiol Renal Physiol* 279:F671–F678, 2000
30. Kaysen GA, Eiserich JP: The role of oxidative stress-altered lipoprotein structure and function and microinflammation on cardiovascular risk in patients with minor renal dysfunction. *J Am Soc Nephrol* 15:538–548, 2004
31. Fleming I, Michaelis UR, Bredenkotter D, *et al*: Endothelium-derived hyperpolarizing factor synthase (cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. *Circ Res* 88:44–51, 2001
32. Fichtlscherer S, Dimmeler S, Breuer S, *et al*: Inhibition of cytochrome P450 2C9 improves endothelium-dependent, nitric oxide-mediated vasodilatation in patients with coronary artery disease. *Circulation* 109:178–183, 2004
33. Passauer J, Pistrosch F, Lassig G, *et al*: Nitric oxide- and EDHF-mediated arteriolar tone in uremia is unaffected by selective inhibition of vascular cytochrome P450 2C9. (in press)