Increased Nitric Oxide Production in Patients with Hypotension during Hemodialysis

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■ *Objective:* To determine the involvement of nitric oxide production in hemodialysis-induced hypotension.

■ *Design:* Examination of nitric oxide synthesis, cyclic guanosine 3′5′-monophosphate (cGMP) levels, and endothelin-1 levels in plasma before and after hemodialysis.

■ *Setting:* Veterans Affairs medical center.

■ *Patients:* 13 patients with end-stage renal failure who were receiving hemodialysis: Six patients had hypotensive episodes during dialysis and 7 did not.

■ *Intervention:* Patients received heparin at a bolus dose of 2000 U at the initiation of dialysis followed by 1000 U/h during 4-hour hemodialysis sessions.

■ *Results:* Nitric oxide production markedly increased during hemodialysis-induced hypotensive episodes; this increase was not seen in patients who did not have a hypotensive episode. In both groups, the plasma cGMP and endothelin-1 levels decreased after hemodialysis. According to multiple regression analysis, standard coefficients of nitric oxide production, plasma cGMP levels, and endothelin-1 levels with mean blood pressure after hemodialysis were −0.743, −0.07, and 0.31, respectively.

■ *Conclusion:* Nitric oxide production increased in patients who had a hypotensive episode during hemodialysis but did not increase in those who did not have a hypotensive episode.

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Hypotension is a major complication of hemodialysis that often requires aggressive resuscitative measures and premature termination of hemodialysis. Heparin is widely used as an anticoagulant during hemodialysis and has been shown to reduce blood pressure in hypertensive humans (1). We have recently shown that heparin promotes vasodilator nitric oxide production and suppresses vasoconstrictor endothelin-1 production by human vascular endothelial cells in culture (2). Elevated levels of endothelin-1 have been reported in hypertensive patients (3, 4). Blocking of this peptide action by a selective antagonist has been reported to reduce blood pressure (5). Nitric oxide promotes the formation of cyclic guanosine 3′5′-monophosphate (cGMP), which causes vasodilatation and inhibits the production of endothelin-1 in aortic endothelial cells (6).

We hypothesized that nitric oxide stimulated by heparin might play a role in vasodilatation and thereby lead to hypotensive episodes during hemodialysis. To test this hypothesis, we measured plasma levels of nitrite (NO_2) and nitrate (NO_3), the products of nitric oxide, and endothelin-1 and cGMP levels in patients who did and did not have hypotensive episodes during hemodialysis sessions in which heparin was used.

Methods

Our study was approved by the institutional ethics committee of the study hospital and followed institutional ethical guidelines. Informed consent was obtained before the initiation of the study.

Study participants included 13 patients with end-stage renal failure who were receiving 4-hour maintenance hemodialysis three times a week. On the basis of their blood pressure responses during hemodialysis, the patients were divided into two groups: Six patients had hypotensive episodes during hemodialysis and 7 did not. Patients who had hypotensive episodes during hemodialysis were defined as those in whom mean arterial pressure decreased more than 20 mm Hg (hypotension occurred 3.7 ± 0.05 hours [mean ± SD] after initiation of dialysis). Renal failure was caused by hypotension in 6 patients, by chronic glomerulonephritis in 5 patients, and by diabetes mellitus in 2 patients. All patients were in stable condition before and after hemodialysis. None of the patients was receiving antihypertensive or other medications that could have confounded the data. The clinical characteristics of both groups are shown in Table 1. No clinical variables differed between patients who did and did not have a hypotensive episode during hemodialysis. All patients were given heparin at a bolus dose of 2000 U at the initiation of dialysis, followed by 1000 U/h.

In all cases, blood samples were obtained when dialysis was initiated. After 4 hours of dialysis, the plasma was quickly separated by centrifugation and was divided into three separate portions for the measurement of nitric oxide synthesis and plasma endothelin-1 and cGMP levels.

Nitric oxide synthesis was determined by measuring the products of nitric oxide, NO_2 and NO_3. For the measurement of NO_2 and NO_3 levels, plasma was quickly deproteinized using 5% trichloroacetic acid and was divided into two aliquots. Because NO_3 is stable in blood, one of the aliquots was evaporated and dissolved in Tris–HCl buffer. Nitrate was converted in the presence of NO_3 reductase. For the measurement of NO_2, Greiss reagents were added to the plasma for stabilization. The samples were then evaporated and dissolved in Tris–HCl buffer. Absorbance was measured at 543 nm by spectrophotometer as previously reported (2). Plasma endothelin-1 levels were measured according to previously described methods (3). A commercial kit (Amersham, Tokyo, Japan) was used to measure plasma cGMP levels after extraction by ethanol.

The statistical significance of differences in the variables between patients with and those without hypotensive episodes was evaluated with the Student *t*-test. Differences in the mean values between the variables before and after hemodialysis in each group were analyzed with two-tailed paired *t*-tests. Multiple regression analysis was done to determine the contribution of

Table 1. Baseline Characteristics of Patients Who Received Maintenance Hemodialysis*

Characteristic	Patients without Hypotension during Dialysis	Patients with Hypotension during Dialysis
Patients (male, female), *n*	7 (6, 1)	6 (6, 0)
Age, *y*	59.9 ± 4.9	67.7 ± 6.0
Duration of dialysis, *mo*	24.4 ± 10.6	26.8 ± 8.2
Weight before dialysis, *kg*	75.9 ± 6.8	81.3 ± 4.0
Weight change with dialysis, *kg*	−2.2 ± 0.37	−2.8 ± 0.46
Systolic blood pressure, *mm Hg*	146.7 ± 8.0	161 ± 11.2
Diastolic blood pressure, *mm Hg*	77 ± 3.6	80 ± 6.7
Mean blood pressure, *mm Hg*	100 ± 3.8	107 ± 6.7
Heart rate, *beats/min*	80.3 ± 3.7	74.3 ± 2.1

* Data are expressed as the mean ± SE.

several factors to mean blood pressure, the change in blood pressure, and increased nitric oxide production.

Results

Plasma levels of NO_2 and NO_3 in both groups of patients are shown in Figure 1. In the patients who had hypotensive episodes during hemodialysis, mean blood pressure decreased from 107 mm Hg to 84 mm Hg ($P < 0.001$; Figure 1, *panel A*), and plasma NO_2 and NO_3 levels increased from 5.8 μM to 19.7 μM ($P < 0.01$; Figure 1, *panel B*). In the patients who did not have hypotensive episodes and whose mean blood pressure did not change (100 ± 14.8 mm Hg), plasma NO_2 and NO_3 levels did not increase. The correlation coefficient for the association between the change in blood pressure and the changes in NO_2 and NO_3 levels was 0.759 ($P < 0.01$). In both groups of patients, plasma levels of cGMP and endothelin-1 decreased after hemodialysis (Figure 1, *panels C and D*). The plasma levels of NO_2 and NO_3 correlated inversely with the mean blood pressure after a 4-hour hemodialysis session ($r = -0.85$; $P < 0.001$). Multiple regression analysis showed that the standard coefficients for the association of nitric oxide production and plasma cGMP and endothelin-1 levels with mean blood pressure after hemodialysis were −0.743 (−0.16), −0.07 (−0.024), and 0.31 (0.47), respectively ($P = 0.0016$ [$P < 0.01$], 0.68 [$P > 0.1$],

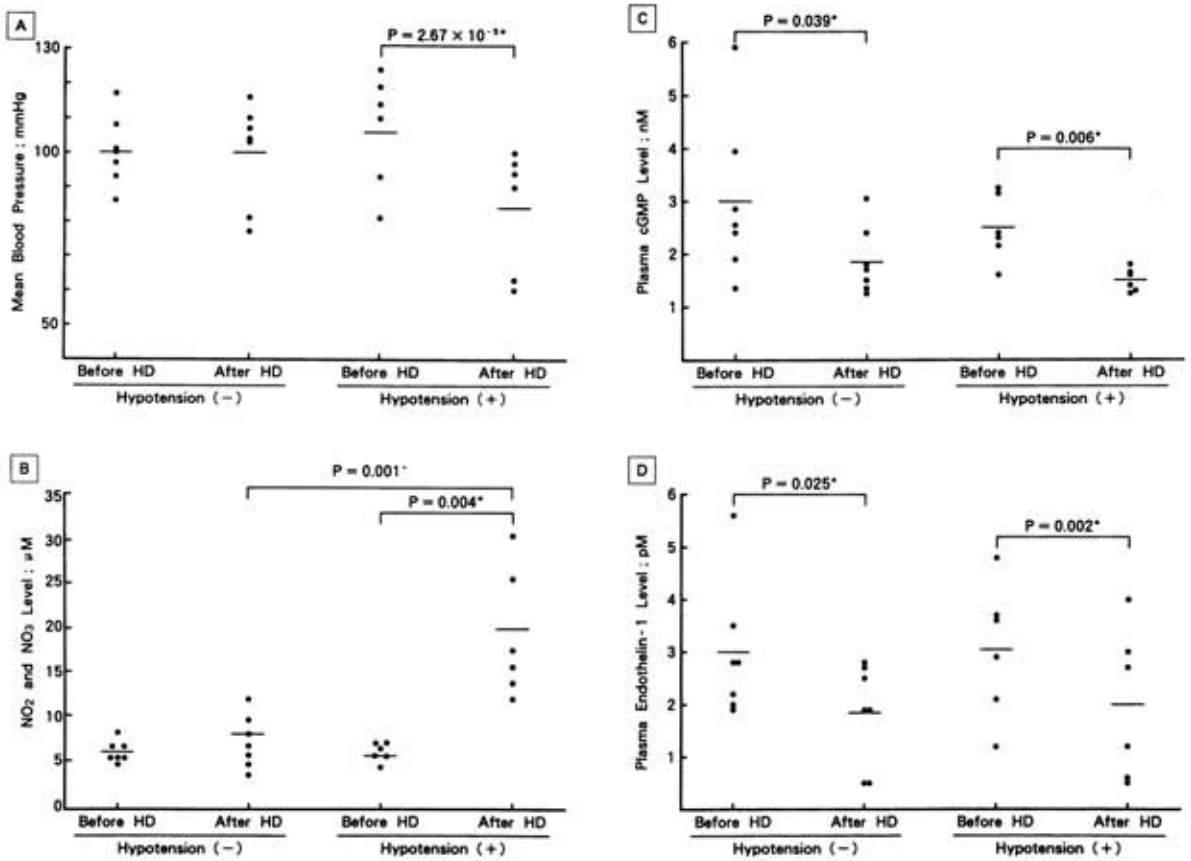

Figure 1. Mean blood pressures (*panel A*); the sum of plasma nitrite (NO_2) and nitrate (NO_3) levels (*panel B*); plasma cyclic guanosine 3′5′-monophosphate (*cGMP*) levels (*panel C*), and plasma endothelin-1 levels (*panel D*) in patients with hypotensive episodes [*hypotension* (+)] and those without hypotensive episodes [*hypotension* (−)] before and after a single 4-hour hemodialysis (*HD*) session. Asterisk indicates the paired *t*-test; plus sign indicates the unpaired Student *t*-test.

and 0.102 [$P > 0.1$], respectively). The correlation coefficient and nonstandard coefficients for the association of baseline systolic blood pressure and baseline heart rate with nitric oxide levels after hemodialysis were −0.129 (−0.532) ($P > 0.1$) and −0.477 (−4.79) ($P > 0.1$), respectively. This suggests that neither was substantially associated with increased nitric oxide production. Dialysate levels of NO_2 and NO_3 in all patients during the observation period were lower than the detection limit of our assay (<0.5 μM). Hemodialysis produced no electrocardiographic evidence of myocardial ischemia, even during hypotensive episodes.

Discussion

Our study was the first in which nitric oxide production was directly measured during hemodialysis. Nitric oxide production at the initiation of hemodialysis did not differ significantly between patients who had and those who did not have a hypotensive episode during hemodialysis. However, nitric oxide production markedly increased during hemodialysis-induced hypotensive episodes, and a strong negative correlation was seen ($P = 0.0003$) between nitric oxide production and mean blood pressure after hemodialysis. This suggests that enhanced nitric oxide biosynthesis may contribute to hemodialysis-induced hypotension. Beasley and Brenner (7) have proposed that hemodialysis-associated hypotension is mediated by the production of cytokine-induced nitric oxide in vascular smooth-muscle cells. Noris and colleagues (8) have reported that in uremic patients, platelets may be a source of increased nitric oxide production, thereby leading to hypotension. We have previously reported that heparin promotes nitric oxide production by human vascular endothelial cells in culture (2). Although all patients received an equal amount of heparin, some patients showed increased plasma nitric oxide production during hypotensive episodes. Therefore, although heparin may not be involved in nitric oxide production, sensitivity to heparin in each patient should be clarified. The exact mechanism of increased nitric oxide production is still unknown. Dialysate levels of NO_2 and NO_3 in all patients during the observation period were below the detection limit of our assay; thus, a reduced blood flow rate does not contribute to greater accumulation of NO_2 and NO_3 in patients with hypotensive episodes during hemodialysis. Plasma levels of cGMP decreased after hemodialysis, a finding similar to those in a previous report (9). This decrease indicates that the vasodepressor effect of nitric oxide may be independent of vascular cGMP levels, although plasma cGMP levels may not necessarily reflect the levels in tissue. Converse and colleagues (10) reported that hemodialysis-induced hypotension is caused by paradoxical withdrawal of sympathetic vasoconstrictors, thereby producing vasodepressor syncope (10). This suggests the presence of nerve firing that triggers cardioinhibitory and vasodepressor responses, which may be a mechanism independent of vascular cGMP (11).

In our study, plasma endothelin-1 levels decreased with hemodialysis; this finding is similar to findings in a previous report (12). Because endothelin-1 is a potent vasoconstrictor peptide, the decrease in its plasma level may cause a reduction in blood pressure. However, multiple regression analysis showed that the contribution of plasma endothelin-1 levels to mean blood pressure after hemodialysis was not statistically significant ($P = 0.1$), suggesting that endothelin-1 in plasma does not contribute much to dialysis-induced hypotension.

In conclusion, nitric oxide production was increased in patients who had hypotensive episodes during hemodialysis compared with those who had no hypotensive episodes. Nitric oxide production was negatively correlated with mean blood pressure after hemodialysis, which suggests that increased nitric oxide production contributes to hemodialysis-induced hypotension.

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