PLASMA NITRIC OXIDE CONCENTRATIONS DURING DIALYSIS INDUCED HYPOTENSION

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ABSTRACT

Objective: We investigated the levels of nitric oxide (NO) and its role in hypotensive episodes in hemodialysis (HD) patients.

Methods: Twelve HD patients (6 male, 6 female, mean age 47.1 ± 2.7 years, mean duration of HD 44.5 ± 8.1 months) were included in the study. Patients who demonstrated ≥20% reduction in mean arterial blood pressure (MAP) during any time period of HD were classified as the hypotensive group, and those who did not demonstrate ≥20% reduction were classified as the nonhypotensive group. NO levels were estimated from NO metabolites (NOx) measured by Greiss reaction in blood samples collected at baseline, 30, 60, 90, 120, 180 and 240 minutes of HD.

Results: Five of 12 patients demonstrated hypotensive episodes during HD. NOx concentrations were similar at the beginning and at the end of HD in hypotensive and nonhypotensive groups. NO concentrations decreased continuously throughout the HD. Percent decline of NO were also similar in both groups during the entire study period. There was no correlation between the NO concentrations and MAP at baseline and at the end of HD.

Conclusion: We suggest that NO could not be responsible for hypotensive episodes in dialysis patients. Further studies should be performed to investigate the factors contributing to hypotensive episodes in these patients so that further therapeutic strategies may be evaluated and applied.

Key Words: Nitric oxide, Hypotension, Hemodialysis.

INTRODUCTION

Hypotension is a significant complication of routine hemodialysis (HD) therapy, occurring both chronically in long-term HD patients and acutely after several hours of HD. Hypotension may be due to insufficiencies in regulation of vascular tone (1,2). Various factors such as uremic autonomic neuropathy, vasodilatory actions of acetate ion, and bioincompatibility of dialysis membranes have been blamed to play role in the development of insufficient vascular tone during HD. In addition, it has been

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suggested that increased nitric oxide (NO) synthesis may be responsible for the acute hypotensive episodes during dialysis (3).

NO is a potent vasodilating substance that plays an important role in the normal regulation of vasomotor tone and blood pressure homeostasis. NO is synthesized in endothelial cells in response to a diverse array of hormonal and physical stimuli by a constitutively expressed enzyme, NO synthase. NO is synthesized from L-arginine, diffuses to the vascular smooth muscle cells and promotes its vasodilatory actions by formation of cyclic guanosine monophosphate (4). Beasly and Brenner have proposed that HD associated hypotension is mediated by the production of NO in vascular smooth muscle cells (5). They proposed that adhesion of monocytes to dialysis membranes causes activation and increased release of cytokines, which enhance NO formation. In vitro studies have shown that the activity of endothelial NO synthase increases when incubated with cuprophan dialysed blood (6).

In addition, heparin, an anticoagulant used during HD, has been shown to reduce blood pressure in hypertensive humans (7). Heparin promotes vasodilator NO production and suppresses vasoconstrictor endothelin-1 in hypertensive patients (8,9).

Although, HD has been suggested to enhance NO production (3,5,6) controversy remains as to whether NO levels are elevated during HD in hypotensive patients or whether NO production correlates with the BP response during HD (10-15). Thus, the role of NO in the regulation of blood pressure (BP) is largely undefined in patients undergoing maintenance HD.

The current study was designed to evaluate dialysis procedure per se on NO production and its impact on BP.

MATERIALS AND METHODS

The study protocol was approved by the Ethical Committee of Marmara University School of Medicine and all subjects provided informed consent for participation in the study.

Twelve HD patients (6 male, 6 female with a mean dialytic age of 44.5 ± 8.1 months, range 12 - 108 months), undergoing 4-h maintenance treatments three times a week were included in the study. All patients had been on chronic HD therapy for over one year at the time of the study, and all were anuric. All patients were in stable condition before and after HD at the time of study. The cause of chronic renal failure was chronic glomerulonephritis in 4 patients, tubulointerstitial nephritis in 2 patients, hypertensive nephrosclerosis in 4 patients, and unknown cause in 2 patients. None of the patients were given any antihypertensive drugs prior to the study. Dialytic therapy was uniform for all patients. They were treated with bicarbonate HD. Dialysis fluid composition included sodium 140 mmol/L, chloride 107.25 mmol/L, potassium 2.0 mmol/L, calcium 1.75 mmol/L, magnesium 0.37 mmol/L, bicarbonate 35.0 mmol/L and acetate 4.0 mmol/L. All patients were treated with cuprophan membrane (Beho, Miandola, Italy). All patients were given 5000 U heparin at the beginning of the dialysis session.

Blood pressure was measured by ambulatory blood pressure monitoring (ABPM) device (Spacelab® 90207, Spacelabs Medical, Redmond, USA) for 48 hours from the start of HD session to the beginning of next dialysis session. Blood pressure measurements were performed every 15 minutes from 07.00 to 23.00 and every 30 minutes from 23.00 ve 07.00. Patients were categorised into "hypotensive" and "nonhypotensive" groups according to BP measurements during the HD. In hypotensive group, patients showed ≥20% reduction in MAP during any time period of dialysis compared to predialysis MAP, whereas nonhypotensive group did not.

Blood samples for determination of NO metabolites (NOx) were collected from the arterial line of dialysis sets into heparinized vacuum tubes on ice at the start (0), 30, 60, 90, 120, 180 and 240 minutes of dialysis session. Plasma was stored at -40°C. Concentration of blood NOx were measured according to the Greiss reaction, after complete reduction of NO_3 to NO_2 by the Cu-coated cadmium method (16).

Statistical Analysis

Data is expressed as mean ± SEM. Differences between the hypotensive and nonhypotensive groups were analysed by Mann-Whitney U test. Wilcoxon Signed Ranks test and ANOVA were used to analyse differences between values in the same groups. Spearman rank correlation analysis was performed to assess the relationship between the NOx and MAP values.

RESULTS

Five of 12 patients demonstrated hypotensive episode(s) during HD. Hypotensive episodes occurred 150 ± 36 minutes after the start of HD. Demographic characteristics of patients are presented on Table I. Age, duration of HD, gender, mean weight change, predialysis SBP, DBP and MAP determined by ABPM, were similar in hypotensive and nonhypotensive groups. The blood pressure response of the two groups during dialysis is shown in Figure 1, and Table II.

Table I.: Baseline characteristics of patients

	Hypotensive group	Nonhypotensive group
Patients (n, M/F)	5 (2/3)	7 (4/3)
Age (years)	44.8 ± 4.6	48.8 ± 3.3
Duration of HD therapy (mo)	48.0 ± 14.7	42.0 ± 8.6
Weight change (kg)	-2.5 ± 0.33	-2.4 ± 0.29
SBP (mm Hg)	146.6 ± 10.2	135.4 ± 9.5
DBP (mm Hg)	90 ± 3.3	80 ± 6.3
MAP (mm Hg)	110.2 ± 4.7	99 ± 7.0

Data is expressed as the mean ± SE. M: male, F: female, HD:hemodialysis, SBP:systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure.

Table II.: MAP response during dialysis in hypotensive and nonhypotensive groups.

Time	Hypotensive group	Nonhypotensive group
n	5	7
Baseline (mm Hg)	110.2 ± 4.7	99.7 ± 7.0
30 min. (mm Hg)	106.2 ± 6.9	93.8 ± 7.0
60 min. (mm Hg)	103.4 ± 7.5	94.1 ± 6.5
90 min. (mm Hg)	93.0 ± 11.0	92.2 ± 7.8
120 min. (mm Hg)	91.6 ± 9.7	92.8 ± 6.2
180 min. (mm Hg)	84.4 ± 6.7	94.8 ± 6.9
240 min. (mm Hg)	81.0 ± 6.4	94.8 ± 7.0

Data is expressed as the mean ± SEM.

NOx concentrations during HD are presented in Figure 2 and Table II. NOx levels were similar at the beginning of dialysis in hypotensive and nonhypotensive groups (103.2 ± 18.5 μmol/L, vs 130.8 ± 11.9 μmol/L; p>0.05). NOx levels continuously declined in both groups throughout the dialysis and plasma (Tables III and IV) / NOx levels were also similar at the end of dialysis in the hypotensive and nonhypotensive groups (53.2 ± 9.8 μmol/L vs 68.8 ± 6.2 μmol/L; p>0.05) (Table III). The mean percent decline in NOx concentrations at the end of dialysis was also similar (39 ± 10.8 % vs 43.2 ± 4.6%) in both groups (Table IV). However, although statistically not significant, mean percent decline of NOx was lower at 120 minutes (32.9% vs 50%) and 180 (32.3% vs 47%) minutes of HD in the hypotensive group compared to nonhypotensive group (Table IV). Mean percent change of NOx in the hypotensive group at 30 minutes of dialysis was significantly less than that in the nonhypotensive group (10.9 ± 7.3% vs —23.1 ± 6.0%, p<0.0025) (Table IV). Hypotensive episodes occured 150±36 minutes after the start of HD. NOx levels at the time of the first hypotensive episode (61.2 ± 12.1 μmol/L) were

Table III.: NOx concentrations (μmol/L) during a dialysis in hypotensive and nonhypotensive groups.

Time	Hypotensive group (n=5)	Nonhypotensive group (n=7)
Baseline (μmol/L)	103.2 ± 18.5	130.8 ± 11.9
30 min. (μmol/L)	97.8 ± 20.5	101.5 ± 11.5
60 min. (μmol/L)	73.2 ± 17.9	82.5 ± 9.6
90 min. (μmol/L)	68.2 ± 19.9 [a]	88.8 ± 9.5[a]
120 min. (μmol/L)	69.2 ± 11.2[a]	65.1 ± 10.6[a]
180 min. (μmol/L)	69.8 ± 14.3[a]	69.0 ± 10.2[a]
240 min. (μmol/L)	53.2 ± 9.8[a]	68.8 ± 6.2[a]

Data is expressed as the mean ± SE. [a] p<0.05 vs baseline.

Table IV.: Percent decline in plasma NOx concentrations in hypotensive and nonhypotensive groups.

Time (min)	Hypotensive group (n=5)	Nonhypotensive group (n=7)
30 min. (%)	10.9 ± 7.3	23.1 ± 6.0*
60 min. (%)	26.2 ± 11.7	34.6 ± 8.2
90 min. (%)	35.5 ± 11.1	32.1 ± 5.2
120 min. (%)	32.9 ± 7.0	50 ± 8.3
180 min. (%)	32.3 ± 12.7	47 ± 7.9
240 min. (%)	39.0 ± 10.8	43.2 ± 4.6

Data is expressed as the mean ± SE. *p<0.005 nonhypotensive vs hypotensive groups.

not different from the mean NOx values 30 minutes prior to hypotensive episodes (72.4 ± 13.6 μmol/L) and 30 minutes after hypotensive episodes (55.2 ± 14.5 μmol/L). The was no correlation between the predialysis NOx concentrations and postdialysis NOx concentrations percent change in NOx concentrations at the end of HD and MAP.

DISCUSSION

NO is an important mediator involved in the regulation of vascular tone. A few studies have suggested that increased NO synthesis - might be responsible for hypotensive episodes during the HD procedure. However, in our study we did not find a correlation between NOx concentrations and BP levels. The result of the present study demonstrates that NOx level either at the time of hypotensive episodes or at the end of HD was not elevated. Moreover, NOx levels at the initiation of HD were statistically different in patients with and without hypotensive episodes during HD.

In the previous studies, increased NO concentrations either at the time of hypotensive episodes or at the end of dialysis have been reported in patients with dialysis induced hypotension (3,14,15). Yokokawa et al, for the first time in in-vivo conditions, reported that NOx levels increased from 5.8 μM to 19.7 μM ($p<0.001$) at the end of HD in hypotensive patients while in nonhypotensive patients this increase was not seen (3). Similarly, Altun et al., demonstrated increased levels of NOx both at the time of hypotensive episodes and at the end of HD in hypotensive patients (14). Heparin has been accused as being responsible for hypotensive episodes during HD treatment by increasing NO production. Mechanism of the hypotensive effects of heparin might be the stimulation of NO synthesis from endothelial cells (17) or inhibition of the production and actions of endothelin-1 (18). Yet it has been difficult to explain the mechanisms of heparin induced hypotension in in-vivo conditions, since the same amount of heparin was used for all HD patients, but hypotension is not observed in all patients. Therefore, the hypotensive effect of heparin has been explained by the differences in the sensitivity of each patient to heparin (3). Noris et al., also suggested that dialysis related hypotension is mediated by increased NO, and explained the mechanism by stimulation of platelet NO synthesis by uremia (19).

On the other hand, several studies could not demonstrate an increase in NOx concentrations at the time of hypotensive episode or at the end of HD in hypotensive patients (11-13). Furthermore, several reports demonstrated that NOx levels consistently decreased during the dialysis (11,12). Decline in NOx levels during HD might be a consequence of decline in intradialytic NO production. Schmidt et al. reported that L-arginine, precursor of NO, declined during HD. In contrast, concentration of asymmetric dimethyl arginine (ADMA), an inhibitor of nitric oxide synthase, was high predialytically, and remained high at the end of HD suggesting that high ADMA levels may inhibit NO synthesis during HD. Total daily NO production by HD patients were also low compared to healthy controls (20). In addition, it has been demonstrated that the diseased kidneys in patients with end stage renal disease have a decreased capacity for synthesizing L-arginine (21). All these data may support the decline in NO levels during the first 120 minutes of HD in our study.

In our study group, hypotensive patients had similar predialytic NOx levels compared to nonhypotensive group. Peer et al., who investigated the NO generation rate during the first 24 hours after HD, found that NO generation rate was higher in hypotension prone patients compared to patients who did not show a hypotensive episode (22). In accordance with this finding, other groups (11,12) reported higher predialytic NO concentrations in patients demonstrating hypotensive episodes during HD, indicating enhanced NO production after each HD session which might be secondary to release of cytokines like IL-1β and TNFα by activated mononuclear cells (5). Madore et al. reported that predialysis end-expiratory NO concentrations correlated inversely with the change in BP during HD. Patients with a decrease in BP during HD had the highest predialytic end-expiratory NO concentrations compared to patients with an increase in BP and with stable BP (11). Likewise, Nishimura et al. also demonstrated that higher predialysis NO_3 levels were associated with a decrease in BP during HD and lower predialysis

NO_3 levels were associated with a rise in BP during dialysis (12). It has also been shown that NO inhibits the function of sympathetic nervous system and elicits hypotension in experimental animals (23).

The studies we have referred to, and our study have several limitations. First, the number of patients involved was small. Second, methodological differences would have resulted in the discrepancies in the results. The determination of NOx concentrations in plasma by Greiss reaction as a reflection NO production during HD may not be a suitable method since NO is a small and rapidly removable molecule by HD. Thus, increase in NOx concentrations may not be detected accurately or the decrease, as in our study, in NOx levels might reflect rapid removal of NO and its end products by dialysis procedure (22). NO measurements in the blood in the outlet of HD lines by NO-selective electrodes or measurement of nitrosylhaemoglobin seem to be more convenient methods to determine intradialytic NO production (24,25).

Regulation of BP control is complex in HD patients and many other factors including paradox removal of reflex vasoconstriction (26), rapid ultrafiltration, acid-base imbalances, and decrease in cardiac output (27)other than NO may also play a role in the regulation of BP in this group of patients.

In conclusion, we could not demonstrate a relation between NO levels and BP levels either predialytically or at the time of hypotensive episodes, suggesting that NO does not play a significant role in dialysis induced hypotensive episodes. Several other factors may contribute to hypotensive episodes of these patients. Further studies should be performed to investigate these factors contributing to hypotensive episodes in these patients so that further therapeutic strategies may be evaluated and applied.

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