THE ROLE OF INTRAVENOUS N-ACETYLCYSTEINE REDUCES PULSE PRESSURE IN PATIENTS WITH CHRONIC KIDNEY DISEASE STAGE 5 DURING HEMODIALYSIS. A RANDOMIZED BLINDED TRIAL

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ABSTRACT

Background *- Patients with chronic kidney disease have increased oxidative stress that cause endothelial dysfunction and show elevated pulse pressure. Whether increased pulse pressure can be prevented by the administration of antioxidants is unknown.* ***Methods and Results*** *– We evaluated the effect of N-acetylcysteine, a thiol-containing antioxidant, on pulse pressure in patients undergoing hemodialysis. A randomized, blinded trial was done in 60 patients (39 male and 21 female) with a mean age of 48.10 ± 11.08 years in N-acetylcysteine group and 52.60 ± 10.30 years in control group (p=0.338). They had been undergoing reguler hemodialysis for 4 hours 2 times weekly in Hemodialysis Unit Dr. Soetomo Teaching Hospital, Surabaya. Patients were randomly assigned either to receive intravenous N-acetylcysteine (Hidonac 5 gram/25 ml solution) or placebo. The primary end point was a composite variable consisting of pulse pressure. A total of 30 (50%) of the 60 hemodialysis patients assigned to N-acetylcysteine group and 30 (50%) of the remaining patients assigned to control group. The significant differences in pulse pressure were detected before and after the intravenous N-acetylcysteine and also than control group.* ***Conclusions****: In hemodialysis patients, treatment with N-acetylcysteine (Hidonac 5 gram/25 ml solution) reduces pulse pressure.*

Keywords *: antioxidants, N-acetylcysteine, pulse pressure*

INTRODUCTION

Cardiovascular disease is the most frequent cause of mortality in chronic renal failure (CRF) (Ivanovski, 2005). Five- and 10-year patient survivals were lower in patients with higher pulse pressure (PP), being vascular disease the main cause of death in this group. The increasing of pulse pressure was associated with higher cardiovascular disease (Fresnedo, 2005). Klassen et al. reported that PP is associated with risk of death and an independent risk factor for increased cardiovascular morbidity and mortality in a large representative sample of patients with chronic kidney disease (CKD) undergoing maintenance hemodialysis. Tozawa et al. reported that in non-diabetic patients on chronic hemodialysis, PP was an independent predictor of total mortality (Fresnedo, 2005). Tozawa et al. in a case–control study concluded that hemodialysis patients had a higher sistolic blood pressure, lower diastolic blood pressure and higher PP values than those control subjects with normal renal function (Fresnedo, 2005). Increased oxidative stress is in part responsible for the endothelial dysfunction observed in patients with CKD stage 5. Impaired endothelial function is associated with abnormalities or arterial wave reflectance, contributing to increase PP. PP itself is known to be an important independent predictor of cardiovascular mortality in patients with CKD stage 5 (Himmelfarb, 2002). Therefore, it is important to identify appropriate treatment measures. The antioxidant N-acetylcysteine (NAC) has been shown to reduce cardiovascular events in hemodialysis patients (Ivanovski, 2005). Scholze's study recently showed the NAC, a thiol-containing antioxidant, is able to significantly reduce PP endpoints in patients with CKD stage 5 (Scholze, 2004). Therefore, antioxidant administration appears to be promising approach.

METHODS

Subjects

This study is a Randomized Blinded Trial (RBT) to find out the role of NAC administration during HD in reducing pulse pressure of patient with CKD stage 5 at Dialysis Unit Dr. Soetomo Teaching Hospital, Surabaya. Subjects of this study are patients with regular hemodialysis (HD) for 4 hours 2 times weekly. The dialysis solutions are bicarbonate/acetate. Kt/V values were measured according to Daugirda's formula. The patients age are between 22-65 years old, Hb > 7 g/dl, albumin ≥ 3 mg/dl, Interdialytic weight gain < 4 kg, mean QB 150-250 ml/minute, had agree and completed the informed consent form.

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Sixty (60) subjects were chosen by simple random sampling followed by random allocation to divide into 30 patients who received NAC intravenously (*Hidonac* 5 g/25 ml solution) intravenously (diluted in 500 ml of 5% glucose solution for 4 hours) and 30 subjects received placebo solution intravenously (placebo in 500 ml of 5% glucose solution for 4 hours). The dosage of NAC is in the range that was associated with a reduction of PP and improvement in endothelial function. PP was measured before and after a single HD session finished and will be compare and analyze in both groups. Blood pressure measurement was performed before, every hour, and after finished HD.

Statistics

The *t test* and *paired t test* was used to compare pulse pressure before and after finished HD between subjects who received NAC intravenously and subjects received placebo solution intravenously. *Two-sided P* values below 0.05 were considered to indicate statistical significance.

RESULTS

The clinical and biochemical characteristics of 60 patients with CKD stage 5 are shown in table 1. Hemodialysis was performed in the absence (placebo control) and presence of NAC (Hidonac) in a Randomized Blinded Trial. Under control conditions and in the presence of NAC, hemodialysis sessions were performed after an essentially equal procedure. In both groups (placebo control versus NAC), there were similar ultrafiltration rate values (placebo, 2.55 ± 0.8; NAC, 2.17 ± 0.98; p = 0.181), interdialytic weight gain (placebo, 2.05 ± 0.8; NAC, 1.82 ± 1.00; p = 0.507), serum albumin concentrations (placebo, 3.85 ± 0.43; NAC, 3.84 ± 0.43; p = 0.267) and hematocrit (placebo 21.53 ± 3.31; NAC, 23.23 ± 3.34; p = 0.265).

Table 1. Clinical and Biochemical Characteristics of 60 Patients With CKD Stage 5

	Placebo	NAC	p
Age (year)	52.60 ± 10.30	48.10 ± 11.08	0.338
Sex : Female	11	10	
Male	19	20	
Interdialytic weight gain (Kg)	2.05 ± 0.8	1.82 ± 1.00	0.507
Months on Hemodialysis	27.67 ± 27.48	44.50 ± 42.06	0.462
Ultra Filtration Rate (L./minute)	2.55 ± 0.8	2.17 ± 0.98	0.181
Hemodialysis Adequacy (Kt/V)	1.63 ± 0.29	1.66 ± 0.31	
Renal Disease (%)			
Diabetes nephropathy	6 (20.00)	2 (6.66)	
Hypertension	14 (46.66)	18 (60)	
Stone	4 (13.33)	8 (26.66)	
Drug Induced	1 (3.33)	2 (6.66)	
Hyperuricemia	1 (3.33)	0 (0)	
Tubulointerstitial nephritis	1 (3.33)	0 (0)	
Polycystic kidney disease	2 (6.66)	0 (0)	
Post Surgical	1 (3.33)	0 (0)	
Dialysat Agent (%)			
Acetate	25 (83.33)	24 (80)	
Bicarbonate	5(16,66)	6(20)	
Hemoglobin (g/dL)	8.15 ± 1.07	7.76 ± 0.62	0.799
Leukocyte (x 10^9/L)	9.06 ± 8.03	7.55 ± 3.70	0.266
Platelets(x 10^9/L)	200.1 ± 66.7	225 ± 101.6	0.278
Serum Homocystein	24.59 ± 11.72	28.09 ± 21.16	0.391
Pack Cell Volume (%)	21.53 ± 3.31	23.23 ± 3.34	0.265
Serum Creatinine (mmol/L)	13.9 ± 5.9	12.44 ± 4.30	0.307
Blood Urea Nitrogen (mmol/L)	73.24 ± 39.49	68.65 ± 17.19	0.245
Albumin (g/L)	3.85 ± 0.43	3.84 ± 0.43	0.267
Serum Calcium (mmol/L)	10.16 ± 1.35	10.25 ± 1.41	0.117
Serum Phosphate (mmol/L)	6.07 ± 2.07	5.86 ± 2.07	0.424
			0.230

Serum Potassium (mmol/L)	4.73 ± 0.96	4.81 ± 0.89	0.677
Serum Sodium (mmol/L)	134.18 ± 3.64	134.18 ± 3.64	

Continous data are shown as mean ± SD. Systolic and diastolic blood pressure and heart rate were measure immediately before, during and after hemodialysis session.

The previous study with intravenous administration of NAC during the hemodialysis session did not show any clinical side effects. In our study, 3 patients (5%) reported clinical side effects during treatment with NAC. Hypotension may be a side effect of NAC administration. In our study, 2 patients (3.3 %) were reported hypotension and 1 patient was urticaria (1.6%). We evaluated the effect of NAC on PP in patients with CKD stage 5. The changes of heart rate, systolic and diastolic blood pressure, and PP are shown in table 2. Under placebo control conditions, PP was significantly increase during a hemodialysis session (84.03 ± 21.26 versus 91.10 ± 20.84 mmHg; p = 0.025), whereas in the presence of NAC, the PP was significantly reduced from 81.97 ± 17.99 to 67.03 ± 23.56 mmHg at the end of the hemodialysis session (p = 0.000). A 10 % decrease in plasma homocysteine (hcy) concentration was associated with a decreased of PP by 1.45 mmHg (Thaha, 2005).

Table 2. Hemodynamic Parameter in 60 patients with CKD Stage 5 Before and After a Hemodialysis Session Under Control Conditions (Placebo) and in the Presence of NAC

Characteristic / Treatment	Before Hemodialysis	After Hemodialysis	p
Heart Rate, bpm			
Placebo	83.93 ± 14.29	88.40 ± 13.31	0.011
NAC	80.96 ± 9.42	86.89 ± 12.95	0.000
Systolic blood Pressure, mmHg			
Placebo	169.40 ± 20.91	176.27 ± 26.91	0.086
NAC	166.33 ± 24.07	154.34 ± 30.04	0.001
Diastolic blood pressure, mmHg			
Placebo	85.70 ± 13.88	85.17 ± 16.00	0.806
NAC	84.37 ± 18.48	87.31 ± 11.09	0.344
Pulse Pressure, mmHg			
Placebo	84.03 ± 21.26	91.10 ± 20.84	0.025
NAC	81.97 ± 17.99	67.03 ± 23.56	0.000

Data are mean ± SD.

DISCUSSION

Patients with CKD stage 5 are at greater risk of developing atherosclerosis than patients with normal kidney function. Patients with end-stage renal disease (ESRD) have up to a 30 times higher risk of cardiovascular-related death than the general population (Friedman, 2001). Consequently, the mortality rate in such patients is substantially higher than in the general population, and deaths are mainly attributable to cardiovascular disease (I vanovski, 2005).

The normal range and the reference values of PP have not been previously reported except those by Asmar et al. These authors showed that 50 mmHg was likely the reference value for PP in 61,724 ambulatory unselected subjects in France. Diagnostic thresholds for clinic PP (>65 mmHg) determined either by adding 2 SD to the

mean or from the 95th percentiles are in close agreement with clinic PP values previously reported to be associated (Fresnedo, 2005).

A wider PP range resulting from a higher systolic blood pressure (SBP) and lower diastolic blood pressure (DBP), correlate with a significant risk of cardiovascular complication in patients with CKD. In CKD stage 5 patients, the increased arterial stiffness is associated with acceleration of the arterial aging process (Blacher, 1999). Increased arterial stiffness, as indicated by increased PP, has been associated with an increased risk for adverse cardiovascular events, including stroke, myocardial infarction, heart failure, cardiovascular death and overall mortality in a number of populations (Fresnedo, 2005).

Oxidative stress damage to endothelial cells is postulated to be of prime importance in the development of fatty streaks, the early lesion of atherosclerosis. Oxidative stress has been identified as one important cause of vascular injury in several studies (Mezzano, 2001). Although oxidative stress seems to be operative also in atherosclerotic disease in unselected populations, it has been documented that oxidative stress is markedly increased in CKD (Tepel, 2000). Several products of oxidative metabolism have been reported to accumulate in CKD. Advanced glycation end products have been studied (Miyata, 1996). A variety of mechanisms have been proposed, by which oxidation products elicit vascular injury (Wolin, 2000). Oxidized low-density lipoproteins are known to accumulate in the arterial intima, inducing several steps of atherogenesis. By the reaction of nitric oxide with reactive oxygen species, peroxynitrite is generated, which in turn exhibit several unfavorable vascular actions, and the availability of nitric oxide is reduced. Last, the increased production of advanced glycation end products adds to the atherogenic potential of renal insufficiency (Tepel, 2003).

Uremic patients are exposed to a number of additional factors. Thus, they suffer from a dysregulation of the immune system and exhibit an excessive generation of reactive oxygen species (ROS). Increased oxidative stress induced by ROS is associated with atherosclerosis and cardiovascular morbidity and mortality in general population and in CRF patients (Ivanovski, 2005). Atherosclerotic lesions were significantly larger in uremic mice than in non-uremic controls. This accelerated atherosclerosis was associated with an increase in *aortic nitrotyrosine expression* and *collagen plaque content* (Ivanovski, 2005).

The vessels elastin is arranged in multiple concentric lamellae interspersed with smooth muscle and collagen. They begin to develop early in fetal life and rates of elastin synthesis in blood vessels increase maximum in perinatal period. Thereafter, rates of elastin synthesis fall rapidly. Turnover of elastin is extremely slow (its half-life is approximately 40 years) and that there is no appreciable synthesis of mature elastin in adult life (Martyn, 2001).

In the vessels, the fatiguing effects of cyclic stress lead to fracture of elastin fibres and the transfer of stress to collagen fibers. Collagen is nearly 1000-fold stiffer than elastin and the gradual loss of elastin is inevitably accompanied by a reduction in vascular compliance. Loss of compliance leads to a rise in PP and an increase in the circumferential stress in the arterial wall. Vascular smooth muscle cells respond to mechanical stretch by synthesizing collagen, which results in thickening of the arterial wall and a further decrease in compliance. A feedback loop is established that tends to maintain higher levels of PP and finally increase cardiovascular disease (Figure 1) (Martyn, 2001).

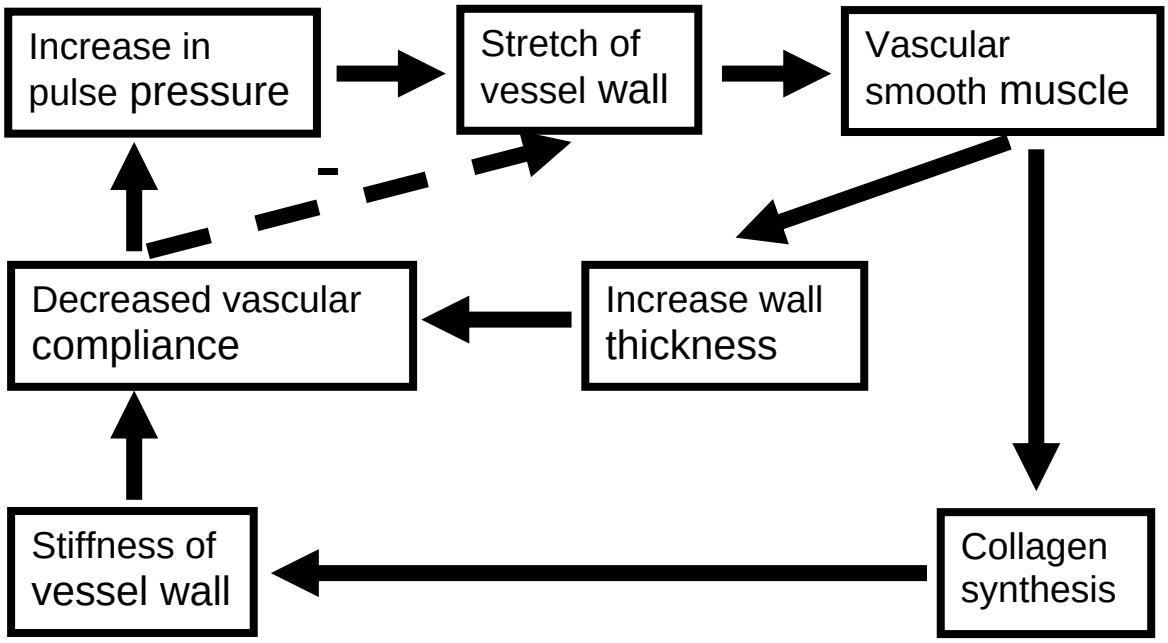

Fig. 1 Inter-relation of vascular compliance, pulse pressure and synthetic activity of vascular smooth muscle cells and collagen

Collagen content was increased in the lesions of uremic mice as compared to control animals (Ivanovski, 2005). Decreased vascular compliance has been recognized as a marker of cardiovascular disease and PP is a powerful independent predictor of cardiovascular events (Martyn, 2001). Recently, beneficial cardiovascular effect has been reported in HD patients treated with another antioxidant, vitamin E. On this background, the beneficial effects of NAC on cardiovascular events might be explained. NAC is a sulfhydryl compound that introduces additional reductive capacity into the oxyreductive metabolism. (Boaz, 2000).

NAC has been shown to increase plasma free hcy, the form removed by the kidney, by breaking the disulfide link of the bound form (Hultberg, 1997). A dose-response curve is apparent with oral doses showing benefits are higher with doses of 1800 mg/day while lower doses often do not respond to even high oral NAC doses (Friedman, 2003). But 5 g of NAC provided to CKD stage 5 patients during dialysis resulted in significantly lower hcy levels and greatly improved endothelial function ($p<0.01$) as measured by fingertip photopletysmograph. At present, the use of high oral doses of NAC may be a potential additional regimen containing the multivitamin approach outlined previously. Patients with impaired kidney function are not likely to benefit from this approach, however, unless NAC is used during dialysis (Guillans, 2004)

NAC, a thiol-containing antioxidant, is currently used therapeutically in several disorders related to oxidative stress. NAC exerts direct and indirect antioxidant activity due to its sulfhydryl group. Moreover, NAC releases cysteine after deacetylation, which in turn increases the formation of reduced glutathione sulfhydryl (GSH) within the intracellular pool of antioxidant molecules. GSH in turn can react with peroxynitrite to form *S*-nitrosothiols, which may prevent the accumulation of peroxynitrite toward the range of toxic levels and protect against nitrosative stress. GSH represents one of the most important natural antioxidant defense systems that decrease early in the course of CKD and progresses with its degree of severity (Ivanovski, 2005). Therefore, it is important to identify appropriate therapeutic measures. The anti-oxidant NAC has been shown to reduce cardiovascular events in HD patients.

NAC treatment inhibited the progression of atherosclerotic lesions and plaque collagen content compared with placebo treatment. In addition, plaques from *NAC-treated uremic* animals showed a significant *decrease in nitrotyrosine expression* whereas the degree of macrophage infiltration was comparable in both uremic groups (Ivanovski, 2005). The antioxidant NAC is capable of *reducing atheroma progression*, in an animal model of *uremia-enhanced atherosclerosis*, probably via a *decrease in oxidative stress*. Liu et al showed that NAC abrogated transforming growth factor (TGF)-stimulated collagen production in fibroblast. NAC also blocked hcy-induced collagen expression in human vascular smooth muscle cells (Ivanovski, 2005). NAC treatment significantly reduced collagen content to levels

that were similar to the ones observed in control animals (Ivanovski, 2005).

Furthermore, uremic mice showed an increase in nitrotyrosine expression in the aorta compared to non-uremic control mice. Such an increase was observed in the atherosclerotic plaques and also in the aortic medial layer in the uremic animals. NAC treatment in uremic mice significantly prevented the observed up-regulation. Ivanovski's study showed that the administration of the antioxidant NAC led to a reduction of atheromatous lesion progression in an animal model of uremia-enhanced atherosclerosis. This reduction was associated with a decrease of plaque collagen content and aortic nitrotyrosine expression, but not with macrophage infiltration (Ivanovski, 2005). The induction of uremia in mice actually led to significant plaque progression in all aortic segments studied, and this enhancement could be prevented by NAC treatment (Ivanovski, 2005).

We investigated the effect of intravenous administration of NAC during a single HD sessions on plasma in a prospective randomized blinded clinical trial design. Our results showed that the administration of NAC could reduce PP to near normal. In patients CKD stage 5, NAC showed antioxidant effect and it can reduce PP via the repairing of arterial stiffness.

CONCLUSION

The findings of our study that a 10% decrease in plasma hcy concentration in patients with CKD stage 5 was associated with a decrease of PP by 1.45 mmHg constitutes an important observation and is in accordance with a recent study showing that acute hyperhomocysteinemia in healthy subjects increases PP.Intravenous administration of NAC reduces PP. Because the effects described here are correlative, additional outcome studies with a different study design may be warranted. However these effects may explain the published beneficial effects of NAC on cardiovascular morbidity in patients with CKD stage 5 and therefore, intravenous administration of NAC during the HD session might be a novel, promising approach to reduce arteriosclerotic risk in patients with CKD stage 5.

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