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Original Article

Effect of Intravenous *N*-acetylcysteine on Plasma Total Homocysteine and Inflammatory Cytokines During High Flux Hemodialysis

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Abstract

Objective: Hyperhomocysteinemia and increased inflammatory cytokines are independent risk factors in patients with renal diseases. *N*-acetylcysteine (NAC) is an antioxidant that is known to decrease inflammatory cytokines and plasma total homocysteine (tHcy). Therefore, the aim of this study was to compare normal saline injection with and without intravenous NAC during hemodialysis (HD) in terms of changes in serum levels of tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), high-sensitivity C-reactive protein (hs-CRP) and plasma tHcy.

Patients and Methods: In total, 43 high flux HD patients were enrolled at a 4-hour HD session and split into two groups, NAC (n=22) and NS (n=21) treatment groups, which received either a normal saline injection with intravenous NAC or without intravenous NAC, respectively. The NS group was divided into two subgroups, one with residual renal function (n=5) and the other with anuria (n=16). The NAC group was also divided into two subgroups, one with residual renal function (n=6) and the other with anuria (n=16). Serum TNF-α, IL-10, hs-CRP and tHcy were measured before and immediately after HD.

Results: There were no significant differences in baseline characteristics, TNF-α, IL-10, hs-CRP, and tHcy levels in intra- and intergroup comparisons. Compared to pre-HD baseline values, plasma tHcy level was lower after HD in the NS group (p<0.001), NS with anuria subgroup (p=0.001), NS with residual renal function subgroup (p=0.034), NAC group (p<0.001), NAC with residual renal function subgroup (p<0.001), and NAC with anuria subgroup (p=0.003). There were no statistically significant differences in plasma tHcy level when the NS group was compared with the NAC group. Plasma tHcy level was significantly lower in the NAC with residual renal

function subgroup compared with the NS group ($p=0.002$), the NAC with residual renal function subgroup compared with the NS with anuria subgroup ($p<0.001$), and the NAC with residual renal function subgroup compared with the NAC with anuria subgroup ($p=0.001$). Moreover, the reduction in plasma tHcy level during HD was greater in the NAC with residual renal function subgroup than in the NAC with anuria subgroup ($p=0.001$).

Conclusion: A normal saline injection is able to decrease plasma tHcy during high flux HD with or without NAC. However, a combination of the two further decreases plasma tHcy in high flux HD patients who still have residual renal function. NAC is only effective when there is residual renal function present to decrease plasma tHcy in high flux HD patients. (*Tzu Chi Med J* 2010;22(2):90–95)

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1. Introduction

Cardiovascular disease (CVD) represents the leading cause of death in hemodialysis (HD) patients (1,2). The increased incidence of CVD in dialysis patients can only be partially explained by an increased prevalence of traditional risk factors, such as hypertension, diabetes mellitus, dyslipidemia, smoking, obesity, and physical inactivity. Additional risk may result from non-traditional factors that are frequently observed in uremic patients, such as hyperhomocysteinemia and inflammation (3,4).

It has been noted that uremic patients are characterized by a state of chronic inflammation (2). A wide array of inflammatory biomarkers, such as high sensitivity C-reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10), are associated with uremic patients (5–7). The combination of an impaired immune response coupled with persistent immune stimulation may play a role in the low-grade systemic inflammation and altered cytokine balance that characterizes the uremic state; this may represent an increased CVD risk (5).

N-acetylcysteine (NAC) is an antioxidant and cytoprotective agent with a scavenging action against reactive oxygen species that also inhibits inflammatory cytokines such as TNF-α and IL-10 (8,9). Intravenous administration of NAC can reduce the plasma total homocysteine (tHcy) level in healthy individuals (10) or in patients undergoing cardiac angiography (11). Intravenous administration of NAC during HD can also normalize plasma homocysteine concentration, which is associated with an improved pulse pressure in uremic patients (12,13). Therefore, the aim of this prospective, double-blinded, randomized study was to compare normal saline injections with or without intravenous NAC in terms of changes in inflammatory cytokines (TNF-α, IL-10), hs-CRP and plasma tHcy levels in HD patients before and after a 4-hour HD session.

2. Patients and methods

2.1. Patients

Forty-three HD patients were using the same high flux polysulfone disposable artificial kidney (FX Class Dialyzer; Fresenius Medical Care, Bad Homburg, Germany). They consisted of 28 men and 15 women, who had a daily oral intake of 5 mg folic acid together with B vitamins complex (25 mg B1, 25 mg B6, and 0.25 mg B12). They were examined during April 2007 at a medical center in Hualien in eastern Taiwan. Patients from our HD program were invited to participate in the study if they were >18 years of age, were receiving standard 4-hour dialysis three times a week using standard bicarbonate dialysate, and had been on dialysis for at least 3 months. Patients were excluded if they had a life expectancy <3 months, malignancy, active infection, a known allergy to NAC or refused/unable to provide informed consent. The Protection of Human Subjects Institutional Review Board at Tzu Chi University and Hospital approved the study. Kt/V was measured according to Daugirdas' formula before dialysis and immediately after dialysis using a formal single-compartment dialysis urea kinetic model.

2.2. Study protocol

Patients were randomly divided into two groups. Patients in the NAC and NS groups received intravenous normal saline with or without NAC, respectively (5 g, 25 mL; Hidonac, Zambon Group SPA, Milan, Italy)

to a total volume of 250 mL during the 4-hour HD session. The NS group was divided into two subgroups according to the daily urine amount. One was the NS with anuria group (16 patients; urine amount <50 mL/day) and the other was the NS with residual renal function group (5 patients; urine amount ≥50 mL/day). The NAC group was also divided into two subgroups according to the daily urine amount. One was the NAC with anuria group (16 patients; urine amount <50 mL/day) and the other was the NAC with residual renal function group (6 patients; urine amount ≥50 mL/day).

2.3. Biochemical investigations

Blood samples were taken from each subject before and at 4 hours after HD. Blood samples of approximately 0.5 mL for measuring the blood cell count (Sysmex K-1000; Sysmex America Inc., Mundelein, IL, USA) and other data were immediately centrifuged at 3000 *g* for 10 minutes. The blood was decanted and separated into two parts. One part was stored at 4°C for biochemical examination within 1 hour of collection by an autoanalyzer (COBAS Integra 800; Roche Diagnostics, Basel, Switzerland). Plasma tHcy was measured by fluorescence polarized light immunoassay after reduction of the oxidized and protein-bound homocysteine to free homocysteine, and subsequent enzymatic conversion to S-adenosyl-L-homocysteine (Abbott IMx; Abbott Diagnostics, Chicago, IL, USA). The second part was stored at –80°C for later analysis of TNF-α and IL-10 concentrations [11,14].

2.4. TNF-α and IL-10 measured by ELISA

TNF-α and IL-10 concentrations in the blood samples were separately measured by two antibody enzyme-linked immunosorbent assays (ELISAs) using a commercial antibody pair, a recombinant standard, and a biotin-streptavidin-peroxidase detection system (Pierce-Endogen, Thermo Fisher Scientific Inc., Rockford, IL, USA) as described previously [11,14]. Blood samples were collected in serum-separated tubes. All reagents, samples, and working standards were brought to room temperature and prepared according to the manufacturer's instructions. The optical density was determined with an automated ELISA reader at wavelengths of 450 nm and 540 nm.

2.5. Statistical analysis

Data are expressed as mean±standard deviation and were tested for normal distribution by Kolmogorov-Smirnov statistics. Categorical variables were analyzed by the χ^2 test. Comparisons between patients were performed using the Student's independent *t* test (two-tailed) for normally distributed data or the Mann-Whitney U test for parameters that presented with non-normal distribution (TNF-α, IL-10, hs-CRP, tHcy). Data were analyzed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) for Windows. A *p* value of less than 0.05 was considered statistically significant.

3. Results

The clinical characteristics of the HD patients are presented in Table 1. The 43 enrolled patients were randomly assigned to the NAC or NS group in a 1:1 ratio. The NAC group consisted of 22 patients and the NS group had 21 patients. The NS with anuria subgroup had 16 patients and the NS with residual renal function subgroup had 5 patients. The NAC with anuria subgroup had 16 patients and the NAC with residual renal function subgroup had 6 patients. There were no intra- and intergroup statistical differences in sex distribution, diabetes, hypertension or biochemical data.

A comparison of laboratory characteristics of inflammation and tHcy levels during HD among the different groups is presented in Table 2. There were no statistically significant differences in serum hs-CRP, TNF-α, IL-10, and tHcy levels in terms of intragroup and intergroup comparisons. Compared to pre-HD baseline values, tHcy level was lower after high flux HD in the NS group ($p<0.001$), the NS with anuria subgroup ($p=0.001$), the NS with residual renal function subgroup ($p=0.034$), the NAC group ($p<0.001$), the NAC with residual renal function subgroup ($p<0.001$), and the NAC with anuria subgroup ($p=0.003$). Plasma tHcy level was significantly lower in the NAC with residual renal function subgroup compared with the NS group ($p=0.002$), the NAC with residual renal function subgroup compared with the NS with anuria subgroup ($p<0.001$), and the NAC with residual renal function subgroup compared with the NAC with anuria subgroup ($p=0.001$). Moreover, the reduction in plasma tHcy level during HD was greater in the NAC with residual renal function subgroup than in the NAC with anuria subgroup ($p=0.001$).

4. Discussion

The results of our study reveal that a normal saline injection, with or without intravenous NAC, can decrease plasma tHcy after a 4-hour high flux HD session in HD patients. There were no statistically significant differences in plasma tHcy between a normal saline injection with or without intravenous NAC. However, the combination of the two further decreases plasma tHcy in high flux HD patients who still have residual renal function.

Table 1 — Baseline characteristics of hemodialysis patients treated with normal saline (NS) with or without intravenous *N*-acetylcysteine (NAC) during hemodialysis*†‡

	NS group (n=21)	NS with anuria group (n=16)	NS with residual renal function group (n=5)	NAC group (n=22)	NAC with anuria group (n=16)	NAC with residual renal function group (n=6)
Sex (male)	15 (71.4)	11 (68.8)	4 (80.0)	13 (59.1)	10 (62.5)	3 (50.0)
Diabetes (yes)	5 (23.8)	4 (25.0)	1 (20.0)	5 (22.7)	4 (25.0)	1 (16.7)
HT (yes)	11 (52.4)	8 (50.0)	3 (60.0)	12 (54.6)	9 (56.3)	3 (50.0)
Age (yr)	55.05±14.87	56.81±11.92	49.40±22.81	56.45±15.67	58.44±16.25	51.17±13.91
Duration of HD (mo)	64.14±34.38	67.44±33.86	53.60±37.77	49.05±30.54	51.75±35.48	41.83±7.49
Pre-HD body weight (kg)	69.27±14.05	71.24±14.75	62.98±10.29	70.58±15.24	69.90±14.68	72.28±17.23
Post-HD body weight (kg)	66.28±13.06	68.08±13.65	60.56±10.04	67.81±14.98	67.18±14.55	69.40±17.36
WBC (×1000/μL)	6.82±2.00	6.56±1.42	7.68±3.36	6.46±1.86	6.75±1.73	5.75±2.16
Hb (g/dL)	10.43±0.84	10.32±0.88	10.78±0.64	9.99±0.99	10.03±1.11	9.88±0.69
Platelets (×1000/μL)	216.05±62.49	221.19±62.56	199.60±66.37	202.81±61.63	201.93±59.32	205.00±29.81
Albumin (g/dL)	4.02±0.36	3.98±0.39	4.16±0.18	4.11±0.74	3.97±0.24	4.48±1.34
AST (IU/L)	20.19±11.11	21.69±12.10	15.40±5.51	16.29±5.52	17.00±5.64	14.50±5.24
ALT (IU/L)	18.33±9.37	19.75±10.08	13.80±5.02	15.86±5.55	15.40±4.85	17.00±7.43
Total cholesterol (mg/dL)	161.62±30.38	158.06±27.81	173.00±38.78	158.29±41.16	160.40±39.90	153.00±47.66
Triglyceride (mg/dL)	177.48±92.69	177.13±95.43	178.60±93.73	161.43±104.27	163.60±103.70	156.00±115.47
BUN (mg/dL)	67.43±17.65	68.81±18.22	63.00±16.78	67.19±18.47	68.47±20.33	64.00±13.81
Creatinine (mg/dL)	12.00±2.75	11.96±2.72	12.14±3.19	11.37±1.85	12.00±2.75	11.83±1.05
Kt/V (Daugirdas)	1.56±0.28	1.55±0.30	1.60±0.20	1.42±0.26	1.45±0.21	1.36±0.37

*Data presented as n (%) or mean±standard deviation; †data expressed as n (%) were analyzed by χ^2 test and data expressed as mean±standard deviation were analyzed by Student's t test or Mann-Whitney U test; ‡there were no statistically significant differences between groups. HT=hypertension; HD=hemodialysis; WBC=white blood cells; Hb=hemoglobin; AST=aspartate aminotransferase; ALT=alanine aminotransferase; BUN=blood urea nitrogen; Kt/V=fractional clearance index for urea.

Inflammation frequently accompanies uremia in patients because of decreased renal clearance of inflammatory cytokines, comorbid conditions such as diabetes and inflammatory kidney disease, and other dialysis-related factors [4]. HD-induced inflammation has been linked to the development of long-term morbidity and an increased risk of cardiovascular mortality in uremic patients [15,16]. CVD is the single largest cause of morbidity and mortality in this population [1,2]. Studied inflammatory markers, including hs-CRP, TNF-α, IL-6 and IL-10, are associated with uremic patients [5–7]. IL-10 appears to play a critical role in suppressing the inflammatory response, and IL-6 and TNF-α seem to have proinflammatory and proatherogenic properties [6]. NAC inhibits phagocyte oxidative responses induced by advanced oxidation protein products and reduces oxidative stress-related inflammation in HD patients [17]. However, our study did not find statistically significant differences in serum hs-CRP, TNF-α and IL-10 levels after a 4-hour HD session when normal saline injections with or without intravenous NAC were compared.

Treatment with folic acid, vitamin B_{12}, vitamin B_6, or a combination can result in a significant decrease in plasma tHcy [18]. Hyperhomocysteinemia is considered to be an independent cardiovascular risk factor in uremic patients [18–20], although the most recent intervention studies utilizing folic acid and B vitamins did not support this hypothesis [21]. The accumulation of homocysteine in blood leads to an intracellular increase of S-adenosylhomocysteine, a powerful competitive methyltransferase inhibitor, which is considered to be a predictor of cardiovascular events [22]. Intravenous administration of NAC during an HD session can normalize plasma tHcy concentration, which is associated with an improved pulse pressure in HD patients [12,13]. However, chronic oral NAC therapy did not significantly reduce tHcy levels in HD patients [23]. Our results also showed that plasma tHcy was decreased after an intravenous NAC injection during the high flux HD session. However, compared with a normal saline injection, there were no significant differences in the decrease in plasma tHcy levels between the two groups. Intravenous NAC administration can reduce plasma tHcy by increasing the excretion of urinary thiols and urinary homocysteine and cysteine in the disulfide form [10,24]. Our results also showed that intravenous NAC administration in

Table 2 — Serum TNF-α, IL-10, hs-CRP, and tHcy levels in hemodialysis patients treated with normal saline (NS) with or without intravenous *N*-acetylcysteine (NAC) during hemodialysis*

	NS group (n=21)	NS with anuria group (n=16)	NS with residual renal function group (n=5)	NAC group (n=22)	NAC with anuria group (n=16)	NAC with residual renal function group (n=6)
TNF-α (pg/mL)						
Pre-HD	104.05±189.40	69.63±122.01	224.51±337.49	83.21±150.16	98.20±176.52	48.23±50.72
Post-HD	97.77±199.16	62.33±123.24	230.68±370.51	77.21±156.61	94.73±184.17	34.66±47.93
Difference	0.80±22.66	–2.66±20.19	6.17±33.11	–5.99±37.01	–3.47±43.30	–13.57±10.37
IL-10 (pg/mL)						
Pre-HD	227.25±267.68	171.03±181.34	452.15±536.89	259.64±341.91	285.86±373.37	141.65±140.36
Post-HD	230.56±272.62	169.59±166.87	474.44±570.34	275.46±299.78	306.26±323.69	136.86±116.16
Difference	3.31±24.52	–1.43±22.02	22.28±33.45	15.82±63.87	20.40±69.98	–4.79±24.20
hs-CRP (mg/dL)						
Pre-HD	0.61±0.78	0.77±0.86	0.16±0.10	0.83±2.01	0.89±2.33	0.69±0.77
Post-HD	0.52±0.62	0.74±0.86	0.19±0.13	0.54±0.93	0.49±0.95	0.67±0.94
Difference	–0.09±0.53	–0.13±0.62	0.03±0.03	–0.29±1.52	–0.40±1.78	–0.02±0.30
tHcy (μmol/L)						
Pre-HD	22.14±3.29	21.84±7.02	20.82±0.23	18.88±4.31	17.82±5.42	20.10±2.41
Post-HD	9.91±4.59†	10.68±4.06†	6.42±4.92†	6.22±4.61†	9.97±2.48†	1.85±0.91†‡§‖
Difference	–12.23±6.16	–11.75±6.37	–14.38±6.64	–12.65±6.35	–7.85±4.33	–18.25±2.13¶

*Data presented as mean±standard deviation; †p<0.05 considered statistically significant before and after HD for within-group differences by Mann-Whitney U test (NS group, p<0.001; NS with anuria subgroup, p=0.001; NS with residual renal function subgroup, p=0.034; NAC group, p<0.001; NAC with anuria subgroup, p=0.003; NAC with residual renal function subgroup, p<0.001); ‡p<0.05 considered statistically significant for NS group compared with NAC with residual renal function subgroup by Mann-Whitney U test (p=0.002); §p<0.05 considered statistically significant for NS with anuria subgroup compared with NAC with residual renal function subgroup by Mann-Whitney U test (p<0.001); ‖p<0.05 considered statistically significant for NAC with anuria subgroup compared with NAC with residual renal function subgroup by Mann-Whitney U test (p=0.001); ¶p<0.05 considered statistically significant for NAC with anuria subgroup compared with NAC with residual renal function subgroup by Mann-Whitney U test (p=0.001). TNF-α=tumor necrosis factor-α; HD=hemodialysis; IL-10=interleukin-10; hs-CRP=high-sensitivity C-reactive protein; tHcy=total homocysteine.

HD patients who have residual renal function further reduced plasma tHcy compared with in anuric HD patients or in those who were given a normal saline injection during a 4-hour high flux HD session. It was also found that normal saline infusion reduces plasma tHcy during a high flux HD session. This may be due to the fact that high flux dialysis itself can lower plasma tHcy in HD patients (25). Further studies are needed to clarify the effect on plasma tHcy of normal saline infusion during high flux HD or low flux HD sessions.

There were some limitations to our study. First, the sample size was small; therefore, a larger study is needed to confirm our findings. Second, this study employed a cross-sectional design with only a 4-hour high flux HD session follow-up. Third, the urinary concentration of tHcy was not measured in this study; such a measurement would confirm that intravenous NAC administration in HD patients who have residual renal function is able to further reduce plasma tHcy by increasing the excretion of urinary homocysteine in the disulfide form. Further studies are needed to confirm the association of NAC and plasma tHcy level in HD patients.

A normal saline injection with or without intravenous NAC during a 4-hour high flux HD session can decrease plasma tHcy in HD patients. However, there were no significant differences in the decreases in plasma tHcy levels between an intravenous NAC injection and a normal saline injection after high flux HD. Intravenous NAC administration in HD patients who have residual renal function was able to further reduce plasma tHcy compared with in anuric HD patients and those who were given a normal saline injection in a 4-hour high flux HD session.

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