

N-acetylcysteine reduces malondialdehyde levels in chronic hemodialysis patients – a pilot study

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Key words

N-acetylcysteine – oxidative stress – hemodialysis – malondialdehyde – chronic renal failure – glutathione – atherosclerosis

Abstract. Background: Oxidative stress has been implicated in the development of endothelial damage in hemodialysis (HD). We have assessed the effects of N-acetylcysteine (NAC), a compound with antioxidant effects, on malondialdehyde (MDA), a marker of oxidative stress on lipid peroxidation. Methods: A clinical trial was conducted in which 24 chronic HD patients were divided into 2 groups according to gender, age, time on HD and cause of renal failure. The NAC group (n = 12) received 600 mg of NAC twice a day for 30 days. The remaining patients constituted the control group (n = 12). MDA levels were measured pre- and post-dialysis at the beginning of the study (baseline) and on day 30 (30 days). Results: Baseline pre- and post-dialysis MDA levels were not different between both groups and were above normal values. A significant decrease was found in the NAC group when either pre- or post-dialysis MDA levels were compared to the corresponding control group levels on day 30 (pre-dialysis NAC vs control group 3.01 ± 0.6 vs 4.5 ± 0.73 mol/l, $p < 0.0001$, post-dialysis NAC vs control group 2.76 ± 0.5 vs 4.39 ± 0.7 mol/l, $p < 0.0001$). Only in the NAC group were pre-dialysis MDA 30-day levels different from pre-dialysis baseline levels (3.01 ± 0.6 vs 5.07 ± 1.6 mol/l, $p < 0.002$). Post-dialysis MDA 30-day concentrations were significantly lower than post-dialysis MDA baseline levels (2.76 ± 0.5 vs 4.32 ± 0.7 mol/l, $p < 0.002$) and pre-dialysis MDA 30-day measurements (2.76 ± 0.5 vs 3.01 ± 0.6 mol/l, $p < 0.011$). Conclusions: MDA levels are elevated in chronic HD patients and are not significantly reduced by HD. NAC significantly reduces malondialdehyde levels in chronic HD patients.

ence of and morbidity and mortality from cardiovascular disease are even higher in chronic renal failure patients, particularly those undergoing hemodialysis (HD), mainly because of arrhythmia, cardiomyopathy, hypertension and coronary artery disease, all entities in direct relationship to atherosclerosis [Levey et al. 1998, Raine et al. 1992]. Several factors can influence the development or aggravate atherosclerosis: dyslipidemia (50 to 75% of HD patients have hypertriglyceridemia), hypertension, diabetes (the main cause of end-stage renal disease in the adult population) [Cheung et al. 2000, Lindner et al. 1974, Wheeler 1997] and elevation of fibrinogen, lipoprotein(a) and homocysteine, all considered important pro-thrombotic molecules [Bostom et al. 1996].

Increasing attention has recently been directed to the role of inflammation in atherogenesis [Ross 1999], and the intervention of reactive oxygen species being generated by activated macrophages in the vessel wall has become recognized [Heinecke 1996, Sies 1997]. In particular, oxidative damage to lipids, proteins and endothelial cells is postulated to be of prime importance in the development of fatty streaks, the early lesions in atherosclerosis [Steinberg et al. 1989]. A number of oxidative stress markers have been identified, including protein oxidation and lipid peroxidation products (particularly in HD patients) in which the extracorporeal circuit of blood plays an important role in the generation of free radical specimens [Deschamps-Latscha and Witko-Sarsat 2001, Drüeke et al. 2001]. We decided to investigate the effects of N-acetylcysteine (NAC), a free radical scavenger that increases glutathione [Afaq et al. 2000, Arouma et al. 1989, Vende-

Introduction

Cardiovascular disease is the main cause of death in the general population. The preva-

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Table 1. Patient characteristics.

	NAC group (n = 12)	Control group (n = 12)
Age (years)	63.17 ± 14.41	65.50 ± 13.17
Time on HD (months)	20.33 ± 18.35	20.83 ± 22.30
Females	4 (33%)	5 (41.7%)
Kt/V	1.2 ± 0.3	1.2 ± 0.3
Diabetics	3 (25%)	3 (25%)
PKD	4 (33.5%)	4 (33.5%)
Glomerulonephritis	4 (33.5%)	4 (33.5%)
Ischemic nephropathy	1 (8%)	1 (8%)
Albumin (g/dl)	3.3 ± 0.3	3.2 ± 0.4
Creatinine (mg/dl)	6.8 ± 1.9	7.2 ± 1.4
Cholesterol (mg/dl)	245 ± 21	238 ± 37
Hematocrit (%)	34.1 ± 3	32.9 ± 4

Results are expressed as the mean ± standard deviation; HD = hemodialysis, PKD = polycystic kidney disease.

miale et al. 2001], on malondialdehyde (MDA), a reactive aldehyde that comes from the oxidation of polyunsaturated fatty acids [Janero 1990, Nielsen et al. 1997] and is determined by the measurement of thiobarbituric acid-reactive substances (TBARS) in plasma [Drücke et al. 2001].

Methods

Patients

Twenty-four chronic HD patients were individually matched into 2 groups according to gender, age, time on HD and cause of renal failure. Both groups were not different according to age, sex, time on HD, Kt/V, primary renal disease (Table 1) and diet. We included patients between 41 and 85 years of age, requiring HD for at least 3 months. Exclusion criteria were acute infection, a history of malignancy or acute ischemic events. Patients from the NAC group received NAC (Acemuk, Betapharm, Augsburg, Germany) 600 mg orally twice a day for 30 consecutive days.

Blood collection and processing

MDA: Blood was processed as described elsewhere [Bird and Draper 1984]. Briefly, TBARS were measured using a modified fluorescence method. To 0.2 ml of plasma, 0.05

ml of 4% (w/v) butylated hydroxytoluene and 0.2 ml of 3% (w/v) sodium dodecyl sulfate were added. After mixing, 2 ml of 0.1 N HCl, 0.3 ml of 10% (w/v) phosphotungstic acid, and 1 ml of 0.7% (w/v) 2-thiobarbituric acid were added. The mixture was heated for 45 minutes in boiling water, and TBARS were extracted into 5 ml of n-butanol. After a brief centrifugation, the fluorescence of the butanol layer was measured at 515 nm excitation and 555 nm emission. The values were expressed as nmoles TBARS (MDA equivalents) per ml of plasma. MDA standards were prepared from 1,1,3,3-tetramethoxypropane (normal: 2 – 3.5 mol/l).

Study design

In this pilot study, NAC, a compound with proven free radical scavenger properties [Afaq et al. 2000, Vendemiale et al. 2001, Arouma et al. 1989], was tested for the first time in a small number of clinically stable, chronic HD patients (n = 24) as an antioxidant agent for a short period of time (30 days). The study was conducted to determine the number of patients necessary to assess the effects of NAC and oxidative stress in HD because, to our knowledge, there are no conclusive positive data in the literature that could allow us to calculate the necessary sample size. Due to the characteristics of the NAC tablet (white, lemon-tasting effervescent capsule), a placebo with similar properties was not available in the local market to conduct a placebo-controlled double-blind intervention trial.

Matching was done individually as follows: patient couples were formed in which sex, age, cause of renal failure and time on HD were similar, and names were replaced by numbers. When the 12 couples were determined, each member of the pair was randomly distributed to 1 of the 2 groups.

At the beginning of the study, blood samples were drawn immediately prior to the initiation of the HD session after an overnight fast (day 0), and they were considered as baseline pre-dialysis determinations. At the end of that first session, another set of blood determinations was collected and named baseline post-dialysis determinations. From that day onward, NAC group patients were started on NAC tablets for 30 consecutive

Table 2. Malondialdehyde measurements in both groups.

	Baseline MDA (2 – 3.5 mol/l)		30-day MDA (2 – 3.5 mol/l)	
	Pre HD	Post HD	Pre HD	Post HD
NAC group	5.07 ± 1.6	4.32 ± 0.7	3.01 ± 0.6	2.76 ± 0.5
Control group	4.67 ± 0.6	4.62 ± 0.9	4.5 ± 0.73	4.39 ± 0.7
p	n.s.	n.s.	< 0.001	< 0.001

Results are expressed as the mean ± standard deviation; MDA = malondialdehyde, pre HD = pre-hemodialysis, post HD = post-hemodialysis, n.s. = non-significant.

days as outlined before. One month later, blood was again drawn immediately prior to the initiation of the HD session at the end of the study (day 30), and samples were labeled as 30-day pre-dialysis determinations. Finally, at the end of the last session, another set of blood determinations was obtained and named 30-day post-dialysis determinations.

Hemodialysis topics

Thrice weekly high-flux HD was performed using biocompatible triacetate cellulose membranes (CT190G, Baxter, McGraw Park, Illinois, USA), reused membranes with a mean QB (blood flow rate) of 380 ± 20 ml/min, QD (dialysate flow rate) of 500 ml/min and a mean HD session of 3.5 ± 0.5 h. All patients received epoetin 4,000 U subcutaneously twice weekly. Two patients from each group had to receive 100 mg of i.v. iron saccharate twice monthly post-dialysis to reach a transferrin saturation between 25 and 50%. All patients were on oral folic acid 10 mg/d and ral methylcobalamin 500 g/d. Patients were not taking other vitamin supplementations.

Ethical issues

All patients had to read and sign an informed consent, which had been previously approved by the Teaching and Research Committee of the Hospital Británico.

Statistical analysis

Results are expressed as mean ± SD. Wilcoxon signed rank test was used to assess inter- and intragroup differences; p values of < 0.05 were considered to be significant.

Results

All results are outlined in Tables 2 and 3. MDA baseline serum concentrations were elevated in both groups (NAC group: 5.07 ± 1.6 vs control group: 4.67 ± 0.6 , $p = \text{n.s.}$) and high-flux HD appeared to be insufficient to remove MDA significantly when baseline post-dialysis measurements were compared to the corresponding pre-dialysis values in each group (Tables 2, 3).

All patients remained in the study. NAC was well tolerated by all patients, and only minor side effects were reported: nausea in 2 patients, abdominal discomfort in 2 and transient dry cough and dizziness in 1.

Four patients (2 from each group) were on intravenous iron therapy. In these patients, baseline MDA levels were not different from the rest of the patients (5.01 and 4.81 mol/l vs 4.72 and 4.96 mol/l). Of note, 30-day MDA post-dialysis measurements were lower in the 2 patients from the NAC group vs the 2 from the control group (3.42 and 3.23 mol/l vs 4.27 and 4.74 mol/l).

Discussion

This pilot study demonstrates that HD patients present with high MDA levels and that HD alone was not capable of removing MDA concentrations efficiently. When MDA intergroup comparisons were made, the NAC group patients displayed significant reductions in lipid peroxidation versus the control group (3.01 ± 0.6 vs 4.5 ± 0.73 mol/l, $p < 0.001$) (Table 2). In NAC intragroup comparisons, patients on NAC showed a significant decrease of 30-day post-dialysis MDA levels when compared to their baseline pre- and post-dialysis and 30-day pre-dialysis values (Table 3).

Table 3. Significant intragroup differences in NAC group.

Malondialdehyde comparisons	Z	p*
Baseline post-hemodialysis vs baseline pre-hemodialysis	-1.647	n.s.
30 days post-hemodialysis vs 30 days pre-hemodialysis	-2.536	< 0.011
30 days post-hemodialysis vs baseline post-hemodialysis	-3.059	< 0.002
30 days pre-hemodialysis vs baseline pre-hemodialysis	-3.059	< 0.002

* = mean comparisons, Wilcoxon signed rank-test; n.s. = non-significant.

Although HD does not have a significant impact on the removal of MDA (Tables 2, 3), NAC administration had an added significant effect as shown when NAC and control groups 30-day post-dialysis levels were compared (2.76 ± 0.5 mol/l vs 4.39 ± 0.7 mol/l, $p < 0.001$). Interestingly, a significant decrease was observed in patients on NAC when 30-day pre-dialysis versus baseline predialysis MDA concentrations were compared (3.01 ± 0.6 vs 5.07 ± 1.6 mol/l, $p < 0.002$), which was even higher after HD (Tables 2, 3), suggesting that MDA levels are mainly reduced through the protective action of NAC against lipid peroxidation.

Oxidative stress is defined as a disruption of the equilibrium between the generation of oxidants and the activity of antioxidant systems (enzymatic antioxidants as endogenous superoxide dismutase, catalase and glutathione peroxidase, and non-enzymatic antioxidants called scavengers such as glutathione, -tocopherol or vitamin E and ascorbic acid or vitamin C) [Deschamps-Latscha and Witko-Sarsat 2001]. Oxidative stress has been reported to occur in HD patients principally due to the activation of polymorphonuclear neutrophils and monocytes by uremic toxins and through the contact of blood with dialysis membranes [Ceballos-Picot et al. 1996, Luciak and Trznadel 1991, Westhuyzen et al. 1995]. Consequently, important quantities of highly reactive species are generated. Oxidative stress is exacerbated not only by the generation of oxygen-reactive species but also by a decreased efficiency of antioxidant systems. Profound deficiencies in the activity of the glutathione system have been reported in HD patients [Ceballos-Picot et al. 1996, Ross et al. 1997].

In this setting, NAC which has been reported to increase glutathione concentrations by providing one of its biosynthetic precursors, cysteine [Afaq et al. 2000, Arouma et al. 1989, Vendemiale et al. 2001], could increase glutathione peroxidase activity, diminishing the peroxidation of lipids as MDA [Cereser et al. 2001, Koh et al. 2000]. Although we have not been able to measure reduced or oxidized glutathione in the present study, there is sufficient evidence in the literature to support our hypothesis with regard to our findings [Afaq et al. 2000, Arouma et al. 1989, Urban et al. 1997, Vendemiale et al. 2001].

NAC, a mucolytic agent with proven antioxidant properties among other properties, was well tolerated by our patients at a dose of 1,200 mg/d. Only minor side effects were reported, as stated above. Although NAC acts on leukocytes, it lacks inhibitory effects on phagocytosis and intracellular killing [De La Fuente and Victor 2001, Paulsen and Vorsgren 1989, Urban et al. 1997]. Thus, the risk of infection is not increased. In this regard, no infectious episodes occurred in the present study.

Even though MDA is not an indicator of oxidation of a specific lipid moiety in plasma but is instead an end product of peroxidation of different polyunsaturated fatty acids originated by free radical species on cellular membranes, it has been used in numerous studies as a marker of in vivo lipid peroxidation and consequently of oxidative stress damage on many cells, mainly erythrocytes and leukocytes [Janero 1990, Nielsen et al. 1997]. MDA interacts with lysine residues in apoB, the protein moiety of low-density lipoproteins [Drüeke et al. 2001]. These protein adducts are found in these oxidized lipoproteins and also in atherosclerotic lesions [Drüeke et al. 2001]. Oxidatively modified compounds enter the vascular subendothelial space and cause injury, especially in the setting of reduced antioxidant defense, to endothelial and smooth muscle cells [Navab et al. 1997, Steinberg 1997].

In chronic renal failure patients and in agreement with our results, MDA levels have been recently reported to be increased [Daschner et al. 1996, Hultqvist et al. 1997], in contrast with some previous studies [Schulz and Schiffl 1995, Taccone-Gallucci et al. 1989]. However, there has been no clear dem-

onstration to date that atherosclerotic lesions in chronic renal failure patients contain oxidized low-density lipoprotein molecules [Drüeke et al. 2001], as has been shown in the general population [Salonen et al. 1992].

Recent data show that intravenous iron therapy increases oxidative stress markers [Zager et al. 2002], but in our 4 patients under iron prescription this phenomenon did not occur. Although the number of patients is too small to draw conclusions from this result, it could be due to the low dose of iron prescribed.

Finally, with respect to a direct relationship between MDA and NAC, it has recently been reported that MDA induces aldehyde reductase gene expression, upregulating the production of lipid peroxides. In turn, NAC suppressed this MDA-induced genetic stimulation [Koh et al. 2000]. To our knowledge, no reports have documented the actions of NAC on MDA in HD patients.

Because this was a pilot study with a small number of patients, conclusions must be made with caution. Bearing this concept in mind and according to the preliminary results, we believe NAC is a well-tolerated drug that may be useful to protect against lipid peroxidation in HD patients, as has been shown by a significant reduction in MDA levels. This agent could be employed to reduce the oxidative stress burden and the atherosclerotic risk in which HD patients live.

To test the results of this pilot study, trials with clinically significant endpoints in which morbidity and mortality are considered, need to be performed before NAC use is recommended for HD patients. A prospective randomized trial including a higher number of patients should be performed.

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