ACTION OF N-ACETYLCYSTEINE ON ASYMMETRIC DIMETHYLARGININE AND ALBUMINURIA IN STAGE 1-4 NON-DIABETIC CHRONIC KIDNEY DISEASE PATIENTS


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ABSTRACT

Background: Uremic patients are in a pro-oxidant state and show an increased level of asymmetric dimethylarginine (ADMA), which is due to increased PRMT1 activity and reduced dimethylarginine dimethylaminohydrolase (DDAH) as degradation enzymes. Reactive oxidant species may play an important role in increasing the action of PRMT1 and in inhibiting the action of DDAH. Albuminuria and ADMA are closely correlated with progression of cardiovascular disease in chronic kidney disease (CKD) patients as well as indicators for decreasing renal function. Although ACEIs and/or ARBs reduced albuminuria in CKD patients, the results are still conflicting. Several factors in these patients may play important roles in the mechanism of albuminuria such as oxidative stress. The antioxidant N-acetylcysteine may prove to have beneficial therapeutic effect, because it can reduce oxidative stress as shown by evidence in humans, and subsequently increase ADMA. The objective of the present study is to explore the contribution of the antioxidant N-acetylcysteine (NAC) to the decrease of ADMA and albuminuria in non-diabetic CKD patients. Material and Methods: Patients with non-DM CKD stage 1–4 with albuminuria were randomized to receive ACEI and/or ARB alone (control group) or with antioxidant NAC 600 mg orally twice a day (treatment group). Observations were performed for 3 months to measure ADMA and albuminuria before and after-treatment. 80 patients in total 40 in the control group and 40 in the treatment group were used. Results: After oral treatment with NAC, the plasma level of ADMA in the treatment group increased from 0.604 µmol/l to 0.689 µmol/l, whereas ADMA level in the control group exhibited a higher increase from 0.561 µmol/l to 0.743 µmol/l. The increases in these groups were significantly different (p < 0.02). Moreover, the level of albuminuria was reduced from 148.12 µg/mg • cr to 132.7 µg/mg • cr in the treatment group, and from 75.25 µg/mg • cr to 71.85 µg/mg • cr in the control group. The difference was significant (p < 0.001). Conclusion: The anti-oxidant N-acetylcysteine can be used as adjuvant therapy to inhibit the progression of CKD in patients by decreasing the ADMA level and albuminuria.

Key words: Chronic kidney disease, reactive oxidant species, asymmetric dimethylarginine, albuminuria, N-acetylcysteine

INTRODUCTION

Chronic kidney disease (CKD) is highly prevalent with an estimated world wide prevalence of 10%. In CKD patients the main cause of death is cardiovascular disease (CVD).[1] The mechanism underlying this relationship is the occurrence of endothelial dysfunction due to reduced nitric oxide (NO) bioavailability associated with atherosclerosis.[2–5] Impairment of NO synthesis in CKD might be due to decreased substrates L-arginine or tetrahydrobiopterin (BH4) and/or inhibition of nitric oxide synthase (NOS), which is required for synthesis of NO. Currently, several studies have revealed that the main cause of NOS pathway disturbance is the presence of asymmetric dimethylarginin (ADMA).[6,7] ADMA is suspected to be a predictor risk of CVD in CKD. It is known that ADMA increases in CKD, even in CKD stage 1.[8–10] There are at least four mechanisms of ADMA increase as follows: i) increased protein methylation by PRMT, ii) increase protein turnover, iii) decreased metabolism by dimethylarginine dimethylaminohydrolase (DDAH) and iv) decreased kidney excretion, but it is assumed that increased protein methylation by PRMT is the main mechanism.
Although the molecular mechanisms of increased activity of PRMT and DDAH down-regulation remain unclear, various studies indicate that oxidative stress is the main cause. Researchers showed that increased oxidative stress in patients with CKD is caused by increased ROS (reactive oxygen species) and decreased antioxidants. Release of NO, a potent vasodilator, into the circulation from endothelial cells, regulates vascular resistance and blood flow into organ tissue. NO can also inhibit the process of monocyte adhesion to endothelial cells, platelet aggregation and vascular smooth muscle cell proliferation. If there is a decrease of NO, endothelial dysfunction and glomerular damage characterized by proteinuria will occur. Persistent proteinuria is generally a marker of kidney damage.

Various meta-analyses indicated that angiotensin converting enzyme inhibitors (ACEIs) and/or angiotensin II receptor blockers (ARBs) are able to inhibit serum creatinine increases, proteinuria and the progression to ESKD. Therefore, they are recommended as standard renoprotective and antiproteinuric therapy. However, both ACEIs and/or ARBs reduce proteinuria by only 23–32% within 1 to 4 months. Thus, most CKD patients are still in a proteinuric state. Since proteinuria reduces the GFR (ml/minute/1.73m²), patients quickly become worse. Therefore, efforts are needed to discover alternative adjunctive therapies.

The antioxidant N-acetylcysteine (NAC) contains thiols groups as synthetic precursors of cysteine and glutathione. NAC is officially indicated for prevention of mucolytic, acetaminophen poisoning and contrast-induced nephropathy. Several studies have examined the effect of NAC in patients with CKD. Antioxidant NAC when given to experimental animals was able to reduce homocysteine and glutathione. NAC in patients with CKD. Antioxidant NAC when given to experimental animals was able to reduce homocysteine and glutathione.

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Table 2. ADMA level elevation in both groups is shown in Figure 2.

Table 1. Clinical and demographic characteristics of treatment and control groups

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Male/Female</td>
<td>9/31</td>
<td>15/25</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>54.2 ±</td>
<td>52.5 ±</td>
<td>0.000**</td>
</tr>
<tr>
<td>Staging of CKD I-II/III-IV</td>
<td>20/20</td>
<td>20/20</td>
<td></td>
</tr>
<tr>
<td>ADMA</td>
<td>0.000**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td></td>
<td>0.000**</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td></td>
<td>0.000**</td>
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</tr>
</tbody>
</table>

Figure 1. Graphic correlation of GFR and ADMA level in CKD patients (n = 80).

Levels of albuminuria were examined before and after NAC treatment. Albuminuria was significantly decreased by 3 µg/mg • cr during 3 months of treatment in the control group (p value; 0.016), while it decreased by 15 µg/mg • cr in the treatment group (p value 0.000) Figure 3.

Table 2. ADMA level comparison in the control group and treatment group

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Treatment Group</th>
<th>Normality</th>
<th>Statistical</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA (µmol/l) Before</td>
<td>0.561</td>
<td>0.604</td>
<td>Normal Distribution</td>
<td>Paired-t</td>
<td>0.001 = S</td>
</tr>
<tr>
<td>ADMA (µmol/l) After</td>
<td>0.743</td>
<td>0.689</td>
<td>Normal Distribution</td>
<td>Paired-t</td>
<td>0.001 = S</td>
</tr>
</tbody>
</table>

Table 3. Albuminuria comparison in both groups

<table>
<thead>
<tr>
<th></th>
<th>Albuminuria (µg/mgCreat)</th>
<th>Statistical</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Analysis</td>
<td>(p &lt; 0.05)</td>
</tr>
<tr>
<td>Control Group</td>
<td>75.25</td>
<td>Wilcoxon</td>
<td>0.016 (Significant)</td>
</tr>
<tr>
<td>Treatment Group</td>
<td>148.12</td>
<td>Wilcoxon</td>
<td>0.000 (Significant)</td>
</tr>
</tbody>
</table>

DISCUSSION

In Table 1, the distribution of CKD stages between the treatment group and control group did not differ significantly. Every 10ml/min/1.73 m² decrease of GFR, will accelerate a GFR decline of 0.38 ± 0.08 ml/min/year, so that if there is a difference among the groups, it can
affect the outcome of therapy. However, since the two groups showed no differences, confounding factors can be minimized.

The results obtained from this study showed a correlation between glomerular filtration rate (GFR) in patients with CKD stage 1–4 and ADMA levels (Figure 1), with a moderate level \( r = 0.537 \) of correlation. This result is similar to several previous studies. Kielstein et al.\(^ {9,24} \) showed that ADMA plasma concentrations in non-diabetic CKD patients were significantly different from those in patients without CKD \( (p < 0.001) \), even in the early stages of CKD. The study was also consistent with Baylis et al.\(^ {7} \) that CKD patients with higher ADMA levels showed a high incidence of CKD progression. In research by Yilmaz et al.,\(^ {9,25} \) there we increased levels of ADMA in non-nephrotic proteinuric patients. An NAC dose of 600 mg bid for 3 months is expected to reduce oxidative stress, which will decrease ADMA levels through reduction of PRMT1 activity, increase DDAH activity and further improve endothelial function reflected by decreased albuminuria. So far, no study has mentioned the effective dose of NAC as an antioxidant in CKD stage I–IV. The NAC dose used in the prevention of contrast induced nephropathy is 1200 mg/day before and after the procedure. Cases of contrast-induced acute kidney injury (CI-AKI) have a similar pathogenesis as CKD. However AKI, mostly occurs transiently, while CKD is a chronic process. Extending the use of NAC for 3 months, will decrease the level of oxidative stress.

The results showed that in the control and treatment groups, increased ADMA levels were 0.182 \( \mu \text{mol/l} \) and 0.086 \( \mu \text{mol/l} \). When compared, the ADMA level increase on the treatment group was 0.15 \( \mu \text{mol/l} \) \( \text{cr} \). In the control group, the decrease was only 3.42 \( \mu \text{mol/g} \) \( \text{cr} \). The decrease of albuminuria in the treatment group was significantly greater \( (p = 0.02) \). This evidence suggests that a decrease in proteinuria was very helpful in slowing the acceleration renal deterioration. A two-fold increase in proteinuria may accelerate a decline in GFR by 0.54 \( \pm \) 0.05 \( \text{ml/min/year} \). The decline in GFR at 10 \( \text{ml/min/1.73} \text{m}^2 \) will accelerate the decline in GFR by 0.38 \( \pm \) 0.08 \( \text{ml/min/year} \).\(^ {19} \) This study revealed that administration of NAC antioxidant at 1200 mg/day can inhibit ADMA level increases and reduce albuminuria in CKD stages 1–4 patients with albuminuria who have received ACEI/ARBs therapy. Possible mechanisms underlying this correlation are decreased oxidative stress, decreased PRMT activity and increased DDAH activity. Thus, decreased ADMA level improved endothelial function and reduced albuminuria. It appears that administration of NAC at 1200 mg/day for 3 months in CKD stages 1–4 patients with albuminuria may inhibit ADMA level increases and reduced albuminuria.

**Conclusion**

The anti-oxidant N-acetylcysteine can be used as adjuvant therapy to inhibit the progression of CKD in patients by decreasing the ADMA level and albuminuria.

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