N-acetylcysteine reduces urinary albumin excretion following contrast administration: evidence of biological effect

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Abstract

Background. There are conflicting results regarding the effectiveness of N-acetylcysteine (NAC) in attenuating contrast-induced nephropathy (CIN). NAC administration independently reduces serum creatinine, potentially confounding studies utilizing creatinine-based endpoints. Albuminuria is a marker of renal injury and spot urine albumin:creatinine ratios (ACR) reflect 24-h urine albumin excretion. We performed a pre-specified secondary analysis from our published negative randomized control trial of NAC for prevention of CIN, to determine if NAC administration reduces albuminuria after contrast exposure following cardiac catheterization.

Methods. We included study patients who had paired urine specimens obtained pre- and post-cardiac catheterization. Baseline characteristics were compared using the chi square test or Mann–Whitney U-test, as appropriate. Changes in ACR were evaluated using binomial exact test. The effect of NAC on post-cardiac catheterization changes in ACR ratio was evaluated by ordinal logistic regression.

Results. A total of 125 patients met inclusion criteria (pre- and post-catheterization urinalysis within 7 days). Baseline characteristics neither differ between NAC and placebo groups, nor were they different from those who were excluded. Among the patients receiving NAC, 10.7% improved their ACR ratio and 7.1% worsened; in contrast, in patients on placebo only 4.3% improved, while 21.7% worsened (P = 0.015). Change in ACR ratio was not associated with change in kidney function as measured by calculated creatinine clearance or GFR.

Conclusions. The results of this analysis suggest NAC may attenuate contrast-induced glomerular or tubular injury, as defined by albumin excretion, and appears to be independent of any effect on creatinine-derived measures of kidney function. Larger studies are required to confirm this observation.

Keywords: N-acetylcysteine; contrast nephropathy-albuminuria

Introduction

Contrast-induced nephropathy (CIN) is associated with increased mortality. N-acetylcysteine (NAC) has been proposed as a preventative treatment for CIN due to its action as an oxygen-free radical scavenger and vasodilator [1,2]. Recent prospective studies examining the potential of NAC to reduce the incidence of CIN have produced conflicting results [3–9]. We recently published a large randomized control trial comparing an intravenous bolus of NAC with placebo in a cohort of patients with impaired kidney function undergoing cardiac catheterization [10]. We did not demonstrate any benefit of NAC in this population according to conventional measures of kidney function using serum creatinine-based equations to estimate GFR. Explanations for our results, as well as limitations of this and other studies, have been published elsewhere [5–9,11]. Multiple meta-analyses of the published studies of oral NAC have also come to different conclusions as to the efficacy of this agent [5–9].

There is an increasing recognition that measurement of serum creatinine does not provide an ideal estimation of renal function, and even equations which estimate kidney function with corrections for age, weight and gender, such as the Modification of Diet in Renal Disease (MDRD) [12] and Cockcroft–Gault [13] have limitations. With regard to the issue of NAC in particular, one study suggests that NAC administration itself temporally reduces serum creatinine, thus calling into question the results of all studies utilizing endpoints derived from measures of serum creatinine as a surrogate marker of renal damage [14].
Albuminuria is a direct consequence of renal glomerular/tubular injury and increases with glomerular dysfunction [15,16]. It is a known marker for progression of chronic renal disease and also a risk factor for cardiovascular disease (CVD) [17]. It also responds to changes in diet, exercise, clinical conditions (such as fever) and medications. Spot urine albumin:creatinine ratios are a reasonable surrogate for 24-h urine albumin excretion rates, though certainly not without limitations [18]. Given that NAC does have properties that should hypothetically attenuate tissue injury [1,2,19], together with the limitations of conventional measures of kidney function [20], we conducted the following planned sub-analysis of albuminuria in our previously published randomized control trial. This analysis reviews the impact of NAC on albumin excretion in the patients who had urine specimens both pre- and post-cardiac catheterization.

Study design and methods

Subjects were drawn from a randomized clinical trial testing intravenous NAC (500 mg) vs placebo for prevention of CIN [10]. For the present analysis, we included all subjects enrolled in the main study in whom paired pre- and post-cardiac catheterization urine samples were available within 3 and 7 days of their procedure. Patients were not included if they did not live local to the hospital so that they could attend for repeat urinalysis. As the study was performed in tertiary centres with multiple referring hospitals throughout the province only one quarter of the original patients were able to attend for follow-up urinalysis for this substudy.

Albumin: creatinine ratio measurement

The urine albumin concentration was measured by immunonephelometry on a Beckman Array 360 system; the urine creatinine concentration was measured by colorimetry (modified Jaffe reaction) on a Hitachi 911 analyser. The ratio of concentrations of albumin to creatinine (ACR) was expressed in mg/mmol. The current British Columbia threshold for an abnormal ACR is 2.0 mg/mmol for males and 2.8 mg/mmol for females. This corresponds to an approximate albumin excretion rate of 20 μg/min or 30 mg/day. For simplicity, and given the male predominance in this study population, the cut-off of 2.0 was identified a priori for all analyses. While ACR is a continuous variable, for the purposes of clinical translation and ease, we selected this threshold for analysis purposes.

Statistical analysis

Baseline characteristics were compared using the chi square test or Mann–Whitney U-test, as appropriate. The comparisons of pre- and post-cardiac catheterization changes in ACR were evaluated using the exact binomial test. The effect of NAC on post-cardiac catheterization changes in ACR was evaluated by ordinal logistic regression and the Fisher Exact test as appropriate. All tests of significance are two-sided and P < 0.05 was chosen as the cut-off for statistical significance.

Results

Of 474 patients enrolled in the main study, 125 had paired pre- and post-procedure urine samples and were included in this analysis (median time to post-catheterization sampling was 5 days). The baseline characteristics of those included in this analysis, receiving either NAC or placebo, did not differ systematically from those who were excluded. The only reason for non-inclusion was that the patients failed to give urine samples within the first week post-catheterization. In order to ensure that there was no systematic bias between those who did and did not give urine samples, we include Table 1 which clearly demonstrates similarity between the two groups. Furthermore, the distribution of ACR and creatinine clearance at baseline as a function of randomization to NAC or placebo showed no significant differences.

Changes in ACR after cardiac catheterization were examined according to treatment assignment. Using the a priori cut-point for ACR of 2, we defined three levels of response: decrease in ACR from ≥2 to <2; no change; increase from <2 to ≥2. Among the patients receiving NAC, 10.7% improved their ACR ratio and 7.1% worsened. In contrast, only 4.3% of those assigned to placebo improved, while 21.7% worsened (P = 0.015). Among 72 patients with normal ACR (<2) at baseline, 14% of those assigned to NAC developed raised ACR (≥2) post-procedure, compared with 35% of those who did not (P = 0.046). Furthermore, of those with elevated ACR at baseline (n = 53), ACR decreased in 22.2% (6/27) of those assigned to NAC, compared with 11.5% (3/26) in the placebo group (Fisher Exact test, P = 0.25) (Figure 1). Change in albuminuria status was not associated with change in kidney function as evaluated by either the Cockcroft–Gault or the MDRD equations (P = 0.69 and P = 1.00, respectively) (Table 2).

The effect of NAC on the change in ACR after cardiac catheterization was evaluated using an ordinal logistic regression model. Using the a priori cut-point for ACR of 2 we defined three levels of response: decrease in ACR from ≥2 to <2; non-change, increase from <2 to >2. The use of NAC was associated with a significant reduction in the risk of increased ACR ratio post-cardiac catheterization (OR 0.31; 95% CI 0.12–0.80). Of the potential confounders age, sex, diabetes mellitus, hypertension, peripheral vascular disease and use of angiotensin converting enzyme inhibitors (ACE-I) or diuretics, only hypertension was significantly associated with an increased risk of elevated ACR post-cardiac catheterization (OR 3.21; 95% CI 1.21–3.62). After adjustment for hypertension, NAC was still associated with a significant reduction in the risk of increased albuminuria post-cardiac catheterization (OR 0.43; 95% CI 0.14–0.91). Though not a pre-specified endpoint, when ACR was treated as a continuous variable, the trend towards the reduction in post-catheterization albuminuria with
NAC persisted \((P = 0.09)\). The data are presented using currently clinically accepted cut-points.

Change in albuminuria status was not associated with change in kidney function as evaluated by Cockcroft–Gault or MDRD equations \((P = 0.69\) and \(P = 1.00\), respectively).

Table 1. Characteristics of patients included and excluded from study population

<table>
<thead>
<tr>
<th></th>
<th>Placebo Included</th>
<th>Placebo Excluded(^a)</th>
<th>Placebo (P)</th>
<th>NAC Included</th>
<th>NAC Excluded(^a)</th>
<th>NAC (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N)</td>
<td>69</td>
<td>176</td>
<td>0.59</td>
<td>56</td>
<td>185</td>
<td>0.49</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>69.5 ± 9.9</td>
<td>70.2 ± 9.3</td>
<td>0.73</td>
<td>71.6 ± 9.8</td>
<td>70.6 ± 10.5</td>
<td>0.27</td>
</tr>
<tr>
<td>Female (%)</td>
<td>36.2</td>
<td>38.6</td>
<td>0.88</td>
<td>33.9</td>
<td>42.2</td>
<td>0.58</td>
</tr>
<tr>
<td>Urgent catheterization (%)</td>
<td>50.8</td>
<td>49.7</td>
<td>0.83</td>
<td>58.5</td>
<td>54.4</td>
<td>0.29</td>
</tr>
<tr>
<td>DM (%)</td>
<td>33.3</td>
<td>41.5</td>
<td>0.83</td>
<td>25.0</td>
<td>32.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>68.1</td>
<td>66.7</td>
<td>0.83</td>
<td>64.3</td>
<td>64.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Dyslipidaemia (%)</td>
<td>66.1</td>
<td>64.5</td>
<td>0.83</td>
<td>58.8</td>
<td>57.6</td>
<td>0.87</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>9.8</td>
<td>9.3</td>
<td>0.83</td>
<td>4.1</td>
<td>13.4</td>
<td>0.07</td>
</tr>
<tr>
<td>PVD (%)</td>
<td>26.7</td>
<td>17.5</td>
<td>0.73</td>
<td>1.00</td>
<td>1.00</td>
<td>0.63</td>
</tr>
<tr>
<td>Ejection fraction (%) (median; 25th, 75th)</td>
<td>48.5 (35.0, 59.8)</td>
<td>50.0 (37.3, 61.0)</td>
<td>0.83</td>
<td>50.5 (42.8, 57.8)</td>
<td>49.0 (35.5, 60.0)</td>
<td>0.76</td>
</tr>
<tr>
<td>Calcium channel blocker use (%)</td>
<td>32.8</td>
<td>34.7</td>
<td>0.83</td>
<td>40.0</td>
<td>35.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Beta blocker use (%)</td>
<td>66.1</td>
<td>67.5</td>
<td>0.83</td>
<td>67.9</td>
<td>66.5</td>
<td>0.84</td>
</tr>
<tr>
<td>ACE-inhibitor use (%)</td>
<td>67.2</td>
<td>71.1</td>
<td>0.83</td>
<td>61.1</td>
<td>70.6</td>
<td>0.19</td>
</tr>
<tr>
<td>Diuretic use (%)</td>
<td>50.7</td>
<td>44.5</td>
<td>0.83</td>
<td>43.6</td>
<td>42.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Extent of disease (%)</td>
<td>10.5</td>
<td>13.0</td>
<td>0.83</td>
<td>10.6</td>
<td>14.9</td>
<td>0.18</td>
</tr>
<tr>
<td>No stenosis &gt;50%</td>
<td>10.5</td>
<td>13.0</td>
<td>0.83</td>
<td>10.6</td>
<td>14.9</td>
<td>0.18</td>
</tr>
<tr>
<td>Single vessel with stenosis &gt;50%</td>
<td>12.3</td>
<td>11.0</td>
<td>0.83</td>
<td>25.5</td>
<td>14.9</td>
<td>0.18</td>
</tr>
<tr>
<td>Two vessels with stenosis &gt;50%</td>
<td>22.8</td>
<td>19.9</td>
<td>0.83</td>
<td>27.7</td>
<td>19.9</td>
<td>0.18</td>
</tr>
<tr>
<td>Three vessels with stenosis &gt;50%</td>
<td>47.4</td>
<td>45.2</td>
<td>0.83</td>
<td>41.8</td>
<td>41.8</td>
<td>0.18</td>
</tr>
<tr>
<td>Left main stem involvement</td>
<td>7.0</td>
<td>11.0</td>
<td>0.83</td>
<td>2.1</td>
<td>5.0</td>
<td>0.83</td>
</tr>
<tr>
<td>Baseline ACR (median; 25th, 75th)</td>
<td>1.9 (0.69, 15)</td>
<td>1.6 (0.75, 10.1)</td>
<td>0.83</td>
<td>1.4 (0.82, 5.2)</td>
<td>2.3 (0.88, 12.2)</td>
<td>0.71</td>
</tr>
<tr>
<td>&gt;5 ml/min decline in CrCl (%)</td>
<td>16.2</td>
<td>22.6</td>
<td>0.83</td>
<td>20.2</td>
<td>22.7</td>
<td>0.73</td>
</tr>
</tbody>
</table>

NAC, N-acetylcysteine; CrCl, Cockcroft–Gault estimated creatinine clearance; ACR, albumin:creatinine ratio.

\(^a\)Patients were excluded if they did not have baseline and follow-up urinalysis within 8 days of cardiac catheterization.

\(^b\)\(P = 0.05\) for comparison between NAC vs placebo in patients included in this substudy.

Fig. 1. This shows the differences in pre- and post-catheterization ACR according to baseline ACR (exact binomial test). Those with baseline ACR <2.0 who received NAC had less propensity to increase ACR >2.0 than those who received placebo. The effect for those with ACR >2.0 at baseline is in the same direction (i.e. beneficial effect of NAC) but does not reach statistical significance due to small numbers. \(P\) values are from the exact binomial test.

Table 2. Baseline kidney function by randomization status

<table>
<thead>
<tr>
<th></th>
<th>NAC (n = 56)</th>
<th>Placebo (n = 69)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cockcroft–Gault creatinine clearance (ml/min) (median; 25th, 75th)</td>
<td>45.2</td>
<td>47.0</td>
<td>0.80(^a)</td>
</tr>
<tr>
<td>MDRD creatinine clearance (ml/min) (median; 25th, 75th)</td>
<td>45.3</td>
<td>44.4</td>
<td>0.76(^a)</td>
</tr>
<tr>
<td>Albumin:creatinine ratio (median; 25th, 75th)</td>
<td>1.49</td>
<td>1.50</td>
<td>0.89(^a)</td>
</tr>
<tr>
<td>Albumin:creatinine ratio ≥2.0 (%)</td>
<td>37.7</td>
<td>48.2</td>
<td>0.24</td>
</tr>
</tbody>
</table>

NAC, N-acetylcysteine; MDRD, Modification of Diet in Renal Disease.

\(^a\)Mann–Whitney U-test.

Discussion

This planned sub-analysis of the largest randomized control trial to date examining the impact of NAC on albuminuria in a high-risk patient population with impaired kidney function reveals some important findings. Firstly, we demonstrate that NAC appears to attenuate the change in ACR in patients undergoing cardiac catheterization irrespective of baseline levels of albumin excretion, at a time point relatively remote.
N-acetylcysteine reduces contrast induced urinary albumin excretion to the administration (mean duration 5 days). This is consistent with the proposed biological actions of NAC on ameliorating oxidative damage at the level of the glomerular apparatus [1,2,15,16]. Secondly, the effect of attenuating albumin excretion was not associated with changes of kidney function, as assessed by derivatives of serum creatinine measurement. This may be due to the fact that creatinine measurement is too insensitive to detect minor amounts of damage to the glomeruli or tubular reabsorption capacity. While NAC administration may itself temporarily reduce serum creatinine, the finding of a reduction in albuminuria in the delayed time period in this study (i.e. greater than 3 days after the procedure), argues against simply an artefactual effect of NAC, given the short half-life of that substance. If albuminuria truly reflects changes in glomerular permeability or tubular damage in the kidney [21], then this sub-analysis adds credence to the hypothesis that NAC may have an impact on this parameter, and thus may be protective against CIN.

The results of this study may help to reconcile previous differences between studies. There is an increasing recognition of how inadequate serum creatinine is as a marker of kidney damage, especially in acute situations over short periods of time [14,20]. Despite the multitude of studies examining NAC in this setting, none has previously examined albuminuria. It is possible that the putative benefits of NAC are better demonstrated by changes in albuminuria than serum creatinine. However, it is also possible that this represents another surrogate marker whose modification may not translate into any clinically meaningful benefit. Although albuminuria and raised serum creatinine are both associated with renal disease they are not necessarily correlated [22]. If the goal of NAC administration is the attenuation of glomerular or tubular injury as a consequence of receiving radiographic contrast, then albuminuria, as a more direct measurement of that damage, may be a more appropriate marker. These findings need to be confirmed and correlated with clinical endpoints in a larger prospective study.

There are several limitations to this study. Firstly, we examined only a subset of the patients from the original study. This is a relative limitation, as there appear to be no systematic differences between those with and without urine collection (Table 1). Of note, this study did not include patients who had albuminuria without renal impairment. Secondly, though urine samples for each patient were analysed in the same laboratory, the test itself (ACR) has limitations: it is subject to variability over time, does not assess total albumin (as would HPLC methods), and has not been standardized [23–25]. However, this would be expected to increase the noise in the measurement and would conservatively bias the results towards the null. Thirdly, we cannot rule out that NAC itself alters the ratio (or its measurement) in the urine, as these experiments have not been done. However, given the short half-life of NAC it is unlikely that NAC would exert an effect at 3–7 days post procedure, when we undertook our follow-up measurements. Furthermore, if NAC lowers the creatinine in urine as it does in serum, then the ratio would be higher, not lower as demonstrated in the results.

Conclusion

This study suggests that NAC may attenuate glomerular or tubular injury as approximated by albumin excretion in a gross method. Further studies are required to confirm this, to examine if other urinary biomarkers, specific for glomerular or tubular damage, are also modified by NAC therapy, and to further explore the effects of NAC on measurements of these urinary markers of damage.

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Conflict of interest statement. None declared.

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