N-Acetylcysteine Effect on Serum Creatinine and Cystatin C Levels in CKD Patients

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Background and objectives: N-acetylcysteine (NAC) has been widely used as a prophylactic therapy for contrast-induced nephropathy (CIN). Its efficacy is controversial because of heterogeneity in study results and because of evidence that NAC can alter serum creatinine levels without affecting glomerular filtration rate. This confounding effect of N-acetylcysteine on serum creatinine has not been rigorously tested, however, in a population at risk for CIN and following doses of NAC currently recommended for prophylaxis of CIN.

Design, setting, participants, & measurements: “Double-dose” NAC was administered in the absence of iodinated contrast media to 29 stage 3 to 5 stable chronic kidney disease patients. Serum creatinine and cystatin C were measured before and 4 h and 48 h after the last dose of NAC.

Results: There was no effect of NAC on either serum creatinine or cystatin C levels.

Conclusion: NAC, in doses currently recommended for prophylaxis of CIN, has no effect on serum creatinine or cystatin C levels. It is therefore unlikely that the heterogeneity seen in clinical trials of NAC prophylaxis for CIN is related to a confounding effect on serum creatinine.

N-Acetylcysteine (NAC) has been reported to protect the kidney from injury induced by contrast media, ischemia, and toxins. In all these studies, glomerular filtration rate (GFR) is the surrogate marker of kidney injury and serum creatinine changes are the measured metric of GFR. Critical to understanding the mechanism of such a beneficial effect of NAC is knowledge of whether NAC can alter the serum level of creatinine independent of a change in GFR. NAC has been reported to decrease serum creatinine levels in subjects with normal kidney function. This reduction in serum creatinine was not accompanied by a change in serum cystatin C levels. This suggests an effect independent of a change in GFR, such as an increase in tubular secretion of creatinine or a decrease in creatinine production (1). In clinical practice, NAC is most often recommended for patients with impaired kidney function scheduled to receive iodinated contrast media. It is therefore important to test whether NAC can have a similar effect, independent of change in GFR, in individuals with impaired kidney function. If so, it is possible that NAC provides no reno-protection and its use for prophylaxis of contrast-induced nephropathy (CIN) should be re-examined.

Few studies have addressed the effect of NAC on serum creatinine independent of a change in GFR. Izzedine et al. studied the effect of NAC on serum creatinine in vitro to exclude a potential analytical interaction between them (2). In the range of NAC concentration between 102 and 600 mg/L, no analytical interference with the measurement of serum creatinine by any of the commonly used analytic reactions was observed. The peak serum concentration of NAC after a 600-mg oral dose was estimated to be 465 mg/L. However, with very high level of NAC (≥50 g/L), the serum creatinine decreases by 50%.

Hoffmann et al. studied the effect of NAC in vivo (1). In this study, 50 healthy volunteers with normal renal function received 600 mg of oral NAC twice a day for a total of four doses. This study suggested a small but significant effect of NAC on serum creatinine independent of GFR, as reflected by the unchanged cystatin C. A number of clinical observations has shown that cystatin C is a more sensitive marker than creatinine for the estimation of GFR (3). The Hoffmann et al. data are difficult to apply to clinical practice, as patients who are likely to receive NAC are not those with normal kidney function. Haase et al. (4) also studied 30 patients with normal kidney function who received intravenous NAC for 24 h in association with cardiac surgery. No change in the ratio of serum creatinine to cystatin C was observed at the end of the 24 h infusion or 48 h after the cessation of the infusion compared with baseline values. In addition, there was no effect on urinary creatinine excretion during the infusion (4). However, in clinical practice, NAC is generally recommended for patients with chronic kidney disease as reflected in a GFR of less than 60 ml/min per 1.73 m². Mainra et al. (5) studied 30 patients with stage 3 chronic kidney disease and administered a single 600 mg dose of NAC. They observed no change in serum creatinine or cystatin C at 4, 24, or 48 h after the dose (5). While this study does not support
the Hoffmann et al. observation, it may be relevant that only a single dose was administered.

Materials and Methods

Patients
A total of 30 patients, age 18 to 89 yr, with chronic kidney disease (estimated GFR (eGFR) ≤ 60 ml/min per 1.73 m²) and a less than 10% difference between the baseline creatinine and the average creatinine values from the previous 6 mo were prospectively enrolled from the kidney clinic of a tertiary care hospital between May 2007 and December 2007. The study was approved by Institutional Review Board at the University of Vermont, and all patients gave written informed consent. Demographic information recorded at baseline included age, gender, serum creatinine, eGFR calculated by the 4-variable Modification of Diet in Renal Disease study equation, and cystatin C. Subjects were excluded if they were unable to give informed written consent or unwilling to return for follow-up blood samples, or developed any condition, which in the judgment of one of the investigators might lead to unstable renal function during the trial. Additionally, they were excluded if they were taking any medications that could interfere with tubular secretion or production of creatinine including gemfibrozil, fenofibrate, trimethoprim, sulfasoxasole, cimetadine, and ranitidine.

Protocol
The design of the protocol was to compare changes in serum creatinine after 4 doses of NAC to baseline values. Serum creatinine and cystatin C were measured in the same blood sample before the first dose of NAC (baseline) and at 4 h and 48 h after taking the last dose of NAC. We used cystatin C and serum creatinine as markers of GFR, rather than 24-h collections for calculation of creatinine clearance. NAC, as a liquid solution, was taken orally as 1200 mg every 12 h for four doses. Compliance with the medication was confirmed verbally.

Serum creatinine was measured using an enzymatic dry slide method (Ortho-Clinical Diagnostics, Rochester, NY). Serum cystatin C was measured using the BNII nephelometer (Dade Behring, Deerfield, IL) using a particle-enhanced immunonephelometric assay (N Latex cystatin C). The same method was used for all patients.

Outcome Measures
The primary outcome was the change in serum creatinine at 4 h after the last dose of NAC compared with the baseline serum creatinine. This time point was identical to the time at which Hoffmann et al. (1) found a significant decrease in serum creatinine. Secondary outcomes included the change in serum creatinine at 48 h following the last dose and changes in cystatin C and the ratio of creatinine to cystatin C at 4 and 48 h after the last dose of NAC.

Statistical Analysis
Paired t tests were used to analyze 4 h and 48 h changes in serum creatinine, cystatin C, and the ratio of serum creatinine to cystatin C compared with baseline values.

Results
Thirty patients started the trial. One patient was dropped because of incomplete data. For the remaining 29 patients, the baseline demographic characteristics are reported in Table 1. Our patients were typical of patients with stage 3 to 5 chronic kidney disease with a mean age of 65 yr. The primary cause of chronic kidney disease was diabetes and hypertension in 38% and 41% of patients, respectively. Subject in stage 3 and 4 CKD were equally represented and the average eGFR was 32 ml/min per 1.73 m².

Serum creatinine and cystatin C levels did not change significantly at either 4 h or 48 h following the last dose of NAC compared with the baseline values (Table 2; Figures 1 and 2). However, a small but statistically significant reduction in the ratio of serum creatinine to cystatin C was observed at 4 h but not 48 h.

Discussion
NAC is a modified form of L-cysteine, an amino acid that is a precursor to reduced glutathione. It is known to be a potent antioxidant that scavenges oxygen-free radicals in the body. It also has vasodilatory properties derived from enhanced nitric oxide availability (6). NAC was introduced to clinical use in 1960s as a mucolytic agent in pulmonary diseases including cystic fibrosis (7). More recently, NAC has been used to prevent acute kidney injury following the administration of iodinated contrast media (CIN). CIN is typically defined as either an

Table 1. Baseline characteristics of study patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Age (yr) (mean ± SD)</td>
<td>65.3 ± 11.7</td>
</tr>
<tr>
<td>Male (%)</td>
<td>59</td>
</tr>
<tr>
<td>Female (%)</td>
<td>41</td>
</tr>
<tr>
<td>Baseline serum creatinine (mg/dl) (mean ± SD)</td>
<td>2.05 ± 0.70</td>
</tr>
<tr>
<td>Baseline estimate glomerular filtration rate (MDRD) (mean ± SD)</td>
<td>32.3 ± 9.8</td>
</tr>
<tr>
<td>Stage 3 CKD (%)</td>
<td>48</td>
</tr>
<tr>
<td>Stage 4 CKD (%)</td>
<td>48</td>
</tr>
<tr>
<td>Stage 5 CKD (%)</td>
<td>4</td>
</tr>
<tr>
<td>Cause of CKD</td>
<td></td>
</tr>
<tr>
<td>diabetic nephropathy (%)</td>
<td>38</td>
</tr>
<tr>
<td>hypertension (%)</td>
<td>41</td>
</tr>
<tr>
<td>miscellaneous (%)</td>
<td>21</td>
</tr>
</tbody>
</table>

CKD, chronic kidney disease.
Table 2. Study results at each time point

<table>
<thead>
<tr>
<th>Metric</th>
<th>Time 0 (baseline)</th>
<th>Time 4 h after Last Dose of NAC</th>
<th>Time 48 h after Last Dose of NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>2.06 ± 0.69</td>
<td>2.02 ± 0.70</td>
<td>2.07 ± 0.75</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>1.73 ± 0.66</td>
<td>1.76 ± 0.62</td>
<td>1.77 ± 0.65</td>
</tr>
<tr>
<td>Ratio serum creatinine/cystatin C</td>
<td>1.22 ± 0.24</td>
<td>1.17 ± 0.21a</td>
<td>1.20 ± 0.28</td>
</tr>
</tbody>
</table>

*aP = 0.0295 by paired t test versus time 0.

Figure 1. Serum creatinine values at time 0 (baseline) and 4 h and 48 h after the last dose of N-acetylcysteine in each of the 29 subjects.

Figure 2. Serum cystatin C values at time 0 (baseline) and 4 h and 48 h after the last dose of N-acetylcysteine in each of the 29 subjects.

absolute (≥44 umol/L) or relative (≥25%) increase in serum creatinine within 48 to 72 h of contrast media exposure. CIN occurs following intravenous and intra-arterial administration of contrast and is associated with both in-hospital and long-term adverse events. Because exposure to contrast media is predictable, CIN prevention is a logical target of many innovative therapeutic interventions.

Tepel et al. was the first to report that NAC reduced the incidence of CIN in patients undergoing contrast-enhanced computed tomography scanning (8). The promising results of this study led to widespread use of NAC to prevent CIN, particularly following intra-arterial administration of CM. Since that seminal study, additional single-center studies have been conducted with mixed results. Additionally, a number of meta-analyses did not uniformly support the efficacy of NAC (9). The dose of NAC used in most of these studies was 600 mg orally twice a day for 2 days (total dose 2400 mg) similar to that reported by Tepel et al. (8).

More recent studies have compared higher doses (1200 mg for four doses) to this standard dose. This “double-dose” NAC was shown to be more efficacious in preventing CIN than the standard dose (10,11). These observations call our attention to the unique aspects of NAC pharmacology, including its low bioavailability (12–14), extensive first pass metabolism (15), and possible dose-dependent antioxidant characteristics (16).

Some CIN prevention trials have reported a decrease in serum creatinine in the group randomized to NAC compared with the control arm. These observations have fueled the speculation regarding an artifactual effect of NAC on serum creatinine. However, these clinical trial observations may be confounded by the administration of large amounts of intravenous fluids as part of the prophylaxis strategy. This explanation, however, is inadequate to explain all of the trial results. For example, Tepel et al. (8) observed a 36-μmol/L decrease in serum creatinine in the NAC arm compared with an 18-μmol/L increase in serum creatinine in the control arm given the same amount of fluid (8). Briguori et al. reported a 5-μmol/L decrease in serum creatinine in patients given 600 mg by 4 doses and a 13-μmol/L decrease in patients given 1200 by 4 doses (10). Both groups received the same intravenous fluid challenge. These results suggest that the combination of NAC and intravenous saline might result in a greater increase in GFR.
than intravenous saline alone. By contrast, the administration of 1200 mg of NAC in two divided doses together with 1 ml/kg per hour of 0.9% saline over a 24-h period did not alter serum creatinine in patients with chronic kidney disease before coronary angiography (17).

The efficacy of NAC for prevention of CIN remains controversial despite the large number of prospective randomized clinical trials. Critical to the analyses of the clinical trial data are whether NAC can alter serum creatinine, the most widely used marker of acute kidney injury, independent of a change in GFR. Creatinine is endogenously produced from the nonenzymatic metabolism of creatine primarily in skeletal muscle. It is freely filtered at the glomerulus and also secreted in the proximal tubules by the organic cation transport system. An effect of NAC either on creatinine production or tubular secretion could alter serum levels without a corresponding change in GFR.

An effect of NAC on creatinine kinase, the enzyme that reversibly phosphorylates creatine, has been reported in animals subjected to oxidative stress (18). Oxidative stress resulted in inhibition of creatine kinase favoring the nonenzymatic conversion of creatine to creatinine (19). The inhibition of creatine kinase by reactive oxygen species is reversed by NAC, theoretically reducing the generation of creatinine under these conditions. Whether such an effect occurs in humans or in the absence of oxidative stress is unknown. Contrast media administration is associated with an increase in urinary markers of oxidative stress (20) and can induce oxidative stress, inhibited by NAC, in proximal tubule cells (Briguori C, personal communication). Whether contrast media causes oxidative stress in skeletal muscle is speculative.

An effect of NAC on tubular secretion could also affect serum creatinine levels. Many pharmacologic agents are known to interfere with tubular secretion of creatinine leading to an increase in serum creatinine levels independent of a change in GFR. One way to look for such an effect is to compare change in serum creatinine with another GFR marker that does not undergo tubular secretion. One such marker is cystatin C, a small peptide produced constitutively by nucleated cells (21). It is eliminated through glomerular filtration and does not undergo tubular secretion (22). Many studies suggest that cystatin C is a more accurate marker of GFR for this reason. A change in serum creatinine in the absence of a change in cystatin C would support the hypothesis that NAC stimulates tubular secretion of creatinine.

In our study, we enrolled patients with chronic kidney disease (stage 3 to 5) and assessed the effects of NAC on renal function in the absence of CM, using two surrogate markers of GFR, serum creatinine and cystatin C. These markers were measured simultaneously before and 4 and 48 h after four 1200 mg doses (4800 mg total dose) of NAC given at 12-h intervals over 48 h.

Our study found that neither serum creatinine nor cystatin C was altered by administration of “double-dose” NAC to patients with chronic kidney disease and moderate to severe decreases in GFR. A small, statistically significant decrease in the ratio of serum creatinine to cystatin C was observed at 4 h but not 48 h. It is unclear whether this small change has any clinical or physiologic significance. Although our results support the observations reported by Mainra, they do not completely refute the observations of Hoffmann et al. (1). Despite using significantly higher doses of NAC compared with Mainra et al. (5) (4800 mg versus 600 mg total dose), we found no change in serum creatinine levels suggesting that tubular secretion of creatinine was not affected. However, the lack of a stimulatory effect on tubular secretion of creatinine might reflect the fact that maximum levels of tubular secretion existed before NAC dosing in these patients with well-established chronic renal insufficiency. Tubular secretion of creatinine is known to increase as the level of serum creatinine increases until a transport maximum is reached (23,24). The serum creatinine at which the transport maximum occurs is unknown and may differ between normal kidneys and diseased kidneys. The Hoffmann et al. (1) patients had a serum creatinine of 88 μmol/L and presumably normal kidneys. Under these conditions, tubular secretion of creatinine was probably not at its maximum. An alternative explanation for the lack of effect of NAC on serum creatinine in our study might be that the dose of NAC used was insufficient to stimulate tubular secretion in these diseased kidneys. Nevertheless, we think that any effect of NAC on serum creatinine seen in clinical trials of CIN prophylaxis is likely to represent a true change in GFR.

Limitations
Our study has several limitations. The sample size is small, raising the possibility of a type II error. Our patients were not undergoing oxidative stress as might occur during contrast media administration. If NAC reduces creatinine generation by protecting the activity of creatine kinase during oxidative stress as suggested by others, our study protocol would not have been adequate to explore this effect. Our patient population is 99% white limiting the generalizability of these observations to other ethnic groups.

Conclusion
Our study did not find any change in serum creatinine after administration of “double-dose” NAC in patients with moderate to severe renal impairment either 4 h or 48 h after the last dose. We did not find any changes in cystatin C levels during these same time periods. We conclude that changes in serum creatinine seen in CIN prophylaxis clinical trials are likely related to changes in GFR and not confounded by effects of NAC independent of GFR.

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Disclosures.
None.

References
1. Hoffmann U, Fischereder M, Kruger B, Drobnick W, Kramer BK: The value of N-acetylcysteine in the preven-


