High Dose of N-Acetylcysteine Prevents Acute Kidney Injury in Chronic Kidney Disease Patients Undergoing Myocardial Revascularization

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Background. The renoprotective effect of N-acetylcysteine in patients undergoing coronary artery bypass graft surgery is controversial.

Methods. We assessed the renoprotective effect of the highest dose of N-acetylcysteine sanctioned for clinical use in a prospective, double-blind, placebo-controlled study including 70 chronic kidney disease patients, stage 3 or 4, who underwent coronary artery bypass graft surgery, on cardiopulmonary bypass (CPB) and off CPB, and were randomly allocated to receive either N-acetylcysteine 150 mg/kg followed by 50 mg/kg for 6 hours in 0.9% saline or only 0.9% saline. Acute kidney injury was defined by the Acute Kidney Injury Network classification.

Results. The incidence of kidney injury was reduced in the N-acetylcysteine group (57.1% versus 28.6%, p = 0.016). Nonuse of N-acetylcysteine (relative risk 3.58, 95% confidence interval: 1.04 to 12.33, p = 0.04) and cardiopulmonary bypass (relative risk 4.55, 95% confidence interval: 1.28 to 16.15, p = 0.02) were independent predictors of kidney injury. In patients treated with CPB, N-acetylcysteine reduced the incidence of kidney injury from 63% to 46%. Oxidative stress was increased in control subjects (p < 0.01) and abolished in patients receiving N-acetylcysteine.

Conclusions. Maximum intravenous doses of N-acetylcysteine reduce the incidence of acute kidney injury in patients with kidney disease undergoing coronary artery bypass graft surgery, abolish oxidative stress, and mitigate the negative effect of CPB on renal function.

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(Fluimucil, Zambon Laboratories, São Paulo, Brazil) 150 mg/kg in 500 mL 0.9% IV saline in 2 hours, started 2 hours before surgery, followed by NAC 50 mg/kg in 500 mL 0.9% IV saline over 6 hours (NAC group, n = 35) or 0.9% IV saline (control group, n = 35) at the same volume. This dose was selected because it is the highest dose sanctioned for clinical use [20–22] and because it has been shown to reduce the oxidative burst response to CPB [23]. Allocation was based on random computer-generated numbers. Patients and investigators were blinded to treatment assignment. Operative risk was assessed by the European System for Cardiac Operative Risk Evaluation (EuroSCORE) [24]. Blood samples were collected 24 hours before surgery and up to 72 hours postoperatively. In the first 50 patients, serum cystatin C, neutrophil gelatinase associated lipocalin (NGAL), and thiobarbituric acid reactive substances were determined at the same intervals.

Serum NGAL (ng/mL) and cystatin C (mg/L) were determined by enzyme-linked immunosorbent assay (BioVendor Human Research and Diagnostic Products, Prague, Czech Republic). Serum thiobarbituric acid reactive substances (nmol/mL) were assessed using the thiobarbituric acid method at the Laboratory of Basic Research in Renal Disease, Nephrology, University of São Paulo Medical School.

Surgical Procedures

Patients received general anesthesia. Anesthesia was induced using weight-related doses of fentanyl, midazolam, etomidate, and pancuronium and was maintained with inhaled isoflurane and fentanyl. The decision to use CPB was left to the discretion of the surgeon. Interventions were performed by the same surgical team. The Acute Physiology and Chronic Health Evaluation II (APACHE II) score [25] was applied for postoperative risk assessment. Patients were managed according to routine hospital guidelines, without interference of the investigators, and followed until discharge or death.

Outcomes

The primary outcome was the incidence of AKI, as defined by the Acute Kidney Injury Network classification [17], stages 1, 2, or 3, in the first 72 hours after surgery. Because the Acute Kidney Injury Network is a relatively new system, we used two other markers of renal damage, serum cystatin C and NGAL, to validate our findings. We measured ROS to verify if changes in renal function would coincide with changes in oxidative stress. The determination of cystatin C, NGAL, and ROS were limited to the first 50 patients.

The secondary outcomes were death by any cause, cardiovascular events (myocardial infarction, stroke, heart failure, life-threatening arrhythmia), and need of dialysis.

Statistical Analysis

Analyses were performed on the intention-to-treat basis using statistical software (SPSS Statistics, version 20.0; IBM, Armonk, NY). Results are expressed as means (SD), median, or percentages. Logarithmic transformation of the data was used before analysis when the distribution of the data was not normal, according to the Kolmogorov-Smirnov test. All analyses were two-tailed. The Student t test, the Mann-Whitney U test, and repeated-measures analysis of variance were used to compare continuous variables, as indicated. Categorical variables were calculated by the χ² test or Fisher exact test. Logistical regression analysis was used to verify the variables independently associated with the outcome.

Results

Preoperative Characteristics

Table 1 shows the preoperative characteristics. The proportion of males was greater in the control group (86% versus 57%, p = 0.02) whereas the left ventricular ejection fraction tended to be higher in the NAC group (p = 0.052). In all other aspects, the groups were well balanced. Creatinine, glomerular filtration rate (control, 44.7 ± 12; NAC, 45.9 ± 9.2; p = 0.64) as well as lipids, urea, and glucose were similar in both groups.

Perioperative Characteristics

We found no differences between groups in intraoperative characteristics except that the number of grafts per patient was increased in patients receiving NAC (p = 0.02; Table 2). The APACHE II index fluid balance and diuresis did not differ in the two groups.

Primary Outcome

During the first 72 hours after surgery, AKI developed in 30 patients (43%), 20 in the control group (57.1%) and 10 in the NAC group (28.6%; p = 0.016; Fig 1); 25 episodes of AKI were classified as stage 1, 4 as stage 2, and 1 as stage 3.

The associations between the primary outcome and a group of variables were explored using a model that included the nonuse of NAC, sex, left ventricular ejection fraction, age, diabetes mellitus, associated cardiovascular disease, and CPB. The nonuse of NAC (relative risk [RR] 3.58, 95% confidence interval: 1.04 to 12.33, p = 0.04) and CPB (RR 4.55, 95% confidence interval: 1.28 to 16.15, p = 0.02) were the sole independent predictors of AKI. Whereas NAC reduced the incidence of AKI, the reverse was observed when patients underwent CPB. The interaction between the nonuse of NAC and CPB is depicted in Table 3. The probability of AKI developing increased from NAC+ and CPB− (8%) to NAC− and CPB+ (63%). The risk of AKI developing was 20-fold higher among patients with the latter characteristic than with the former (p = 0.008). Among patients treated with CPB, the use of NAC was associated with a fall in the incidence of AKI from 63% to 41%.

C-reactive protein increased with time in both groups (p < 0.001), and did not differ between groups (p = 0.61).
Cystatin C, NGAL, and Thiobarbituric Acid Reactive Substances

For the first 50 patients, we evaluated the associations between AKI and other markers of renal damage and oxidative stress. Cystatin C and NGAL were higher in the control group (Figs 2 and 3, respectively), but whereas cystatin C increased in both groups ($p < 0.05$), NGAL levels increased only in the control group ($p < 0.01$), and remained unchanged in patients receiving NAC ($p = 0.71$). The effect of NAC on the incidence of AKI in this restricted group was similar to that observed in the totality of the 70 patients (62% versus 25%, $p = 0.02$). The ROS levels increased in the control group ($p = 0.031$) but remained unchanged in patients receiving NAC ($p = 0.62$; Fig 4). Table 4 shows the actual data pertaining to Figures 2 through 4.

### Secondary Outcomes

Six patients (8.6%) died: 2 in the NAC group (5.7%) and 4 in the control group (11.4%; $p = 0.97$). Two deaths were caused by myocardial infarction; 4 deaths were caused by infection. Twelve cardiovascular events occurred in 11 patients (15.7%), 8 events in the control group (23%) and 4 in the NAC group (11%; $p = 0.67$). There were 10 myocardial infarctions and 2 strokes. No patient needed reoperation or dialysis.
Comment

In this study, we showed that maximum doses of NAC sanctioned for human use reduced the incidence of AKI among patients with moderate chronic kidney disease undergoing elective CABG. The nonuse of NAC was an independent predictor of AKI (RR 3.58, \(p = 0.04\)) together with CPB (RR 4.55, \(p = 0.02\)). The data suggest that the nonuse of NAC and CPB interact to increase the probability of AKI and that NAC attenuates the negative effects of CPB on renal function. To our knowledge, this is the first report using the Acute Kidney Injury Network criterion to define AKI in cardiac surgery.

The antioxidant, antiinflammatory, and vasodilatory properties of NAC are the basis for its clinical use\[26, 27\]. However, the renoprotective effect of NAC remains controversial. Although in animal models the results have been promising \[28, 29\], in cardiovascular surgery, the results have been generally negative. Considering the controversial nature of the subject, we compared our data with those reported in prospective, randomized, double-blind studies that used intravenous NAC for patients undergoing cardiovascular surgery (Table 5).

Of nine investigations, in only one was a positive effect of NAC documented. Fisher and coworkers \[16\] administered NAC in doses close to those used in the present work and found a reduction in serum creatinine compared with control subjects.

In eight studies, the effect of NAC did not differ from placebo. In three studies \[30–32\], the doses of NAC administered were lower than in the other studies.

### Table 3. Interaction Between Use of N-Acetylcysteine and Cardiopulmonary Bypass on Incidence of Acute Kidney Injury

<table>
<thead>
<tr>
<th>AKI Yes</th>
<th>AKI No</th>
<th>Total</th>
<th>OR</th>
<th>(p) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAC yes, CPB no</td>
<td>1 (8%)</td>
<td>12 (92%)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>NAC yes, CPB yes</td>
<td>9 (41%)</td>
<td>13 (59%)</td>
<td>22</td>
<td>8.3</td>
</tr>
<tr>
<td>NAC no, CPB no</td>
<td>5 (45%)</td>
<td>6 (55%)</td>
<td>11</td>
<td>10.0</td>
</tr>
<tr>
<td>NAC no, CPB yes</td>
<td>15 (63%)</td>
<td>9 (37%)</td>
<td>24</td>
<td>20.0</td>
</tr>
</tbody>
</table>

AKI = acute kidney injury; CPB = cardiopulmonary bypass; NAC = N-acetylcysteine; OR = odds ratio.
Table 5. Systemic N-Acetylcysteine and Renal Function in Cardiovascular Surgery: Prospective, Randomized, Double-Blind Studies

<table>
<thead>
<tr>
<th>First Author [Reference]</th>
<th>n</th>
<th>Inclusion Criteria</th>
<th>Doses of NAC</th>
<th>Outcome</th>
<th>Need of Dialysis (n)</th>
<th>Effect on Renal Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burns [30], JAMA 2005</td>
<td>295</td>
<td>CABG, DM, CKD, &gt;69 years, heart failure</td>
<td>600 mg IV 4x</td>
<td>25% increase in serum CR up to 5Ω PO</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>Fischer [16], Curr Med Res Opin 2005</td>
<td>40</td>
<td>CABG, normal renal function CPB</td>
<td>100 mg/kg IV (load); 20 mg/kg/h IV up to 24 h</td>
<td>Increase in serum CR up to 24 h</td>
<td>Zero</td>
<td>Lower serum CR with treatment</td>
</tr>
<tr>
<td>Ristikankare [39], Br J Anesth 2006</td>
<td>80</td>
<td>CABG, CKD, CPB</td>
<td>150 mg/kg IV (load); 20 mg/kg IV</td>
<td>Increase in CR, NAG, cystatin C</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Hynninen [40], Anesth Analg 2006</td>
<td>70</td>
<td>Abdominal aortic aneurysm, normal renal function</td>
<td>150 mg/kg IV (load); 150 mg/kg/24 h IV</td>
<td>Increase in CR, NAG, cystatin C</td>
<td>Zero</td>
<td>None</td>
</tr>
<tr>
<td>Macedo [32], Nephrol Dial Transplant 2006</td>
<td>42</td>
<td>Abdominal aortic aneurysm, stable renal function</td>
<td>1,200 mg PO 2x day preop; 600 mg IV 2x day up to 48 h</td>
<td>CR ≥25% up to 2Ω PO</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Sisillo [31], Crit Care Med 2008</td>
<td>254</td>
<td>CKD</td>
<td>1,200 mg IV (load); 1,200 mg IV 8/8 h up to 24 h</td>
<td>CR &gt;25%</td>
<td>16</td>
<td>Nonsignificant protective effect ($p = 0.06$)</td>
</tr>
<tr>
<td>El-Hamamsy [34], J Thorac Cardiovasc Surg 2007</td>
<td>100</td>
<td>Consecutive</td>
<td>600 mg PO 2x day 24 h preop; 150 mg/kg IV (load); 12.5 mg/kg/h up to 24 h</td>
<td>Increase in serum CR</td>
<td>Not reported</td>
<td>None</td>
</tr>
<tr>
<td>Haase [41], Crit Care Med 2007</td>
<td>60</td>
<td>High risk for AKI; CPB</td>
<td>300 mg/kg IV up to 24 h</td>
<td>Increase in serum CR up to 5Ω PO</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>Wijeysundera [35], Can J Anesth 2007</td>
<td>177</td>
<td>CKD</td>
<td>100 mg/kg bolus (load); 20 mg/kg/h IV up to 4 h</td>
<td>Percentual changes in GFR up to 72 h PO</td>
<td>4</td>
<td>No effect on renal function. Reduction in overall mortality ($p = 0.007$)</td>
</tr>
<tr>
<td>Santana-Santos (this study), 2013</td>
<td>70</td>
<td>CKD, CABG, CPB in 66%</td>
<td>150 mg/kg IV 2h (load) before surgery; 50 mg/kg IV up to 6 h</td>
<td>AKIN 1, 2, or 3</td>
<td>Zero</td>
<td>Reduction in incidence of AKI ($p = 0.016$)</td>
</tr>
</tbody>
</table>

AKI = acute kidney injury; AKIN = Acute Kidney Injury Network; CABG = coronary artery bypass graft surgery; CPB = cardiopulmonary bypass; CKD = chronic kidney disease; CR = creatinine; DM = diabetes mellitus; GFR = glomerular filtration rate; IV = intravenous; NAG = N-acetyl-β-D-glucosaminidase; PO = orally; preop = preoperatively.
Low-dose treatment was reported to be beneficial for renal injury associated with radiocontrast [10, 33], but for patients undergoing CABG, low-dose treatment may be insufficient to counteract the greater oxidative stress. In these three cases, the negative results may be explained by the use of insufficient doses of NAC.

Five investigations administered higher doses of NAC. In two, the criteria used to characterize AKI were serum creatinine [34] and the Cockcroft-Gault equation [35], which uses creatinine in its formula. It is now well established that creatinine is a relatively insensitive marker of AKI [7, 36, 37]. The criterion used in our work is more precise because it includes alterations in urinary volume [38]. It is conceivable that those negative results were influenced by the low sensitivity of the tests.

We are left with three studies [39–41] that merit a detailed discussion. In the first 50 patients in our series, we included two independent markers of renal injury, cystatin C and NGAL. Both markers were increased in the control group in comparison with the NAC group. In contrast, in the three studies mentioned above [39–41], the researchers did not find any effect of NAC on cystatin levels. Moreover, urinary N-acetyl-β-D-glucosaminidase (NAG), a marker of tubular injury, was not influenced by NAC in two of the studies [39, 40]. The role of NAG in the diagnosis of renal damage, however, is equivocal because it increases in association with both AKI and cardiac surgery [42].

The renoprotective effect of NAC is attributed to its ability to attenuate the oxidative stress burst associated with cardiac surgery and CPB [23, 29]. In none of the nine studies discussed above did the investigators evaluate the impact of NAC on oxidative stress. In the present investigation, we not only addressed this problem, but we also offered evidence that NAC abolishes the increase in circulating ROS observed in the control group, thus giving a plausible explanation for the renoprotective effect.

Study Limitations
The number of patients was small, and this was a single-center investigation. The groups were mismatched by sex. Only 50 patients were studied in relation to oxidative stress, cystatin-C, and NGAL. The incidence of severe AKI in our sample was small (1 of 30), and we still are ignorant of whether NAC will be effective in a population with a higher incidence of AKI stage 3.

In conclusion, the maximum intravenous dose of NAC reduces the incidence of AKI in patients with preexisting chronic kidney disease undergoing CABG, abolishes oxidative stress, and mitigates the negative effect of CPB on renal function.

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References