**N-acetylcysteine prevents reactive oxygen species–mediated myocardial stress in patients undergoing cardiac surgery: Results of a randomized, double-blind, placebo-controlled clinical trial**

Paschalis Tossios, MD*  
Wilhelm Bloch, MD  
Astrid Huebner*  
M. Reza Raji, MD*  
Fotini Dodos, MD  
Oliver Klass, MD  
Michael Suedkamp, MD*  
Stefan-Mario Kasper, MD  
Martin Hellmich, PhD  
Uwe Mehlhorn, MD

**Objective:** Reactive oxygen species have been shown to contribute to myocardial stress in patients undergoing cardiac surgery, as demonstrated by myocardial 8-iso-prostaglandin-F$_2$α and nitrotyrosine formation. We hypothesized that the reactive oxygen species scavenger N-acetylcysteine attenuates reactive oxygen species–mediated myocardial stress in patients undergoing cardiac surgery.

**Methods:** Forty patients undergoing coronary artery surgery (mean age ± SD, 66 ± 9 years; 9 women and 31 men) were randomized to receive either N-acetylcysteine (100 mg/kg into cardiopulmonary bypass prime followed by infusion at 20 mg·kg$^{-1}$·h$^{-1}$, n = 20) or placebo (n = 20). Patients and clinical staff were blinded to group assignment. Transmural left ventricular biopsy specimens collected before and at the end of cardiopulmonary bypass were subjected to immunocytochemical staining against 8-iso-prostaglandin-F$_2$α (primary measure) as an indicator for reactive oxygen species–mediated lipid peroxidation and nitrotyrosine (coprimary measure) as a marker for peroxynitrite-mediated tissue injury. Cardiomyocyte staining was quantitatively determined by using densitometry (in gray units). Global left ventricular function was measured on the basis of fractional area of contraction by using transesophageal echocardiography.

**Results:** Patient characteristics in both groups were comparable. The change in left ventricular cardiomyocyte staining (end of cardiopulmonary bypass – before cardiopulmonary bypass) differed significantly between groups for both primary measures: 8-iso-prostaglandin-F$_2$α, −1.8 ± 7.5 gray units (mean ± SD, N-acetylcysteine group) versus 5.0 ± 4.1 gray units (placebo group; 95% confidence interval, 2.6-11.0, $P = .003$); nitrotyrosine, −6.4 ± 10.0 gray units (N-acetylcysteine group) versus 9.2 ± 8.4 gray units (placebo group; 95% confidence interval, 9.4-21.7, $P < .001$). Hemodynamics and clinical outcomes were comparable in both groups.

**Conclusions:** Reactive oxygen species scavenging with N-acetylcysteine attenuates myocardial oxidative stress in the hearts of patients subjected to cardiopulmonary bypass and cardioplegic arrest.
R eactive oxygen species (ROS), such as hydroxyl radical (OH·), superoxide radical (O2−), hydrogen peroxide (H2O2), and peroxynitrite (ONOO−), the product of nitric oxide (NO) binding to O2−, are thought to mediate the reperfusion injury that characterizes metabolic, structural, and functional (stunning) myocardial damage associated with experimental myocardial ischemia and reperfusion.1–3 Clinical studies have provided indirect evidence for ROS-mediated stress, such as malondialdehyde and lipid hydroxyperoxide determination in the blood of patients with acute myocardial infarction4 or those subjected to percutaneous transluminal coronary angioplasty5,6 or coronary artery bypass surgery.7 We have recently provided direct evidence for ROS-mediated myocardial stress associated with ischemia and reperfusion because we demonstrated both 8-iso-prostaglandin-F2α and nitrotyrosine formation in left ventricular (LV) biopsy specimens of patients subjected to cardioplegic arrest.8 8-Iso-prostaglandin-F2α is the stable end product of arachidonic acid oxidation generated by ROS attacks on membrane phospholipids.6,9 Nitrotyrosine represents the stable end product of cell membrane protein-bound tyrosine nitration by ONOO−.10,11 These data indicate the clinical relevance of ROS-mediated oxidative stress associated with ischemia and reperfusion and imply a rational basis for ROS scavengers as adjuncts to reperfusion strategies.

Although numerous experimental investigations have demonstrated that ROS scavenging attenuates myocardial ischemia-reperfusion injury,1 only a few clinical studies have addressed their ability to ameliorate myocardial injury associated with cardiopulmonary bypass (CPB) and cardioplegic arrest. For example, pretreatment with allopurinol either alone or in combination with vitamins E and C has been suggested to improve patient outcome after coronary artery surgery by reducing the level of plasma ROS activity7,12 or to improve neurocardiac protection in high-risk pediatric cardiac surgery with deep hypothermic circulatory arrest.13 Menasche and colleagues14 showed that the ROS-scavenging iron chelator deferoxamine reduced the polymorphonuclear neutrophil oxidative responsiveness, and Andersen and associates15 demonstrated that the antioxidant and ROS scavenger N-acetylcysteine (NAC) reduced the neutrophil oxidative burst response in patients subjected to CPB and cardioplegic arrest. However, none of these studies directly investigated the effect of ROS scavenging on myocardial tissue alterations.

Therefore the purpose of our randomized, double-blind, controlled clinical trial was to compare the effects of NAC versus placebo on myocardial 8-iso-prostaglandin-F2α and nitrotyrosine formation as indicators for direct ROS-mediated myocardial alterations in the hearts of patients subjected to coronary artery surgery during CPB.8 We chose to use NAC, a precursor of reduced glutathione, because of its properties to both enhance intracellular glutathione synthesis and directly scavenge ROS.16 NAC has been in clinical use for more than 30 years, primarily as a mucolytic, and is the gold standard for treatment of acetaminophen poisoning.16 In addition, NAC has recently been shown to reduce ROS-induced renal dysfunction in patients subjected to radiographic contrast agents.17

Materials and Methods

Patients
After approval by the University of Cologne Human Ethics Committee, written informed consent was obtained from each patient during the preoperative interview. Forty patients (9 women and 31 men) scheduled for elective or urgent coronary artery bypass surgery (Table 1) were randomized into either the NAC group (n = 20) or the placebo group (n = 20) according to a computergenerated allocation list (randomly permuted blocks of random size) provided by the Department for Medical Statistics, Informatics, and Epidemiology, University of Cologne. NAC (Flumucil) and placebo were supplied in identical-looking glass vials containing either 5 g of NAC per 50 mL or isotonic sodium chloride solution. The vials were sequentially numbered according to the random list and were supplied by the Pharmacy of the University of Cologne. Patients in the NAC group received 100 mg of NAC per kilogram of body weight into the CPB prime, followed by intravenous infusion at 20 mg of NAC per kilogram of body weight per hour until the end of CPB, a regimen similar to that clinically used by Andersen and associates.15 The rationale for administering NAC into the CPB prime and throughout CPB as opposed to adding it to cardioplegia alone was 2-fold: first, we administered NAC before cardioplegia because of its property to enhance intracellular glutathione synthesis,16 and second, we intended to have systemic NAC concentrations at the time of massive ROS production (ie, myocardial reperfusion after aortic cross-clamp release) because of NAC’s property to directly scavenge ROS.16 Patients in the placebo group received equivalent amounts of placebo. The flow chart depicting the trial phases according to the Consolidated Standards of Reporting Trials (CONSORT) statement18 is shown in Figure 1. Patients were subjected to CPB at 32°C to 34°C, the aorta was crossclamped, and myocardial revascularization was performed during cardioplegic arrest by using single-shot, antegrade, cold (4°C) crystalloid Bretschneider cardioplegia (Custodiol, Dr Köhler Chemie).

Clinical Protocol
After anesthesia induction and standard hemodynamic monitoring, including Swan-Ganz pulmonary artery catheterization, a 5-MHz transesophageal echocardiography (TEE) probe was placed to provide a LV short-axis image at the midpapillary level. From the TEE recordings, we derived the fractional area of contraction (FAC) as a measure of LV ejection fraction.8,19 Before cannulation for CPB, we recorded baseline measurements of all hemodynamic parameters, including cardiac output and a 1-minute TEE reading. At 10 to 15 minutes after separation from CPB, all hemodynamic and TEE measurements were repeated.

1514 The Journal of Thoracic and Cardiovascular Surgery • November 2003
LV Biopsy Specimens

Before CPB initiation, we collected a transmural biopsy specimen from a fat-free area of the LV anterior wall by using a 14-gauge biopsy needle (Gallini). A second LV biopsy specimen was taken at the end of the extracorporeal circulation period before weaning from CPB. All LV biopsy specimens were placed in 4% parafor-
maldehyde for 4 hours and then rinsed in 0.1 mol/L phosphate-buffered saline (PBS) for 24 hours, followed by storage for 12 hours in PBS solution with 18% sucrose for cryoprotection and frozen at −80°C.

Immunocytochemistry

Before immunohistochemical examination, 7-μm slices from the biopsy specimens were placed in a bathing solution of 3% H2O2 and methanol for 20 minutes, and then cells were lysed with 0.25% Triton-X 100 in 0.5 mol/L ammonium chloride. Thereafter, specimens were treated with 5% bovine serum albumin solution in 0.05 mol/L Tris-buffered saline. Before each step, the sections were rinsed 3 times in 0.05 mol/L Tris-buffered saline buffer. For nitrotyrosine staining, we used a monoclonal mouse anti-nitrotyrosine antibody (1:400, Calbiochem) and a secondary goat anti-mouse antibody (1:400, DAKO). For 8-iso-prostaglandin-F2α detection, a polyclonal goat anti-8-Epi-PGF2α antibody (1:1500, Oxford Biomedical Research) and a secondary rabbit anti-goat antibody (1:400, DAKO) were used. A streptavidin–horseradish peroxidase complex was then applied as a detection system (1:150) for 1 hour. Finally, staining was developed for 10 to 20 minutes with 3,3-diaminobenzidine tetrahydrochloride in 0.1 mol/L PBS.

8-Isoprostaglandin-F2α and Nitrotyrosine Television Densitometry

All LV biopsy slices were incubated and stored under identical conditions. For quantitative intensity analyses of 8-iso-prostaglandin-F2α and nitrotyrosine immunostaining in cardiomyocytes, we measured the gray values of 30 cardiomyocytes from 6 randomly selected areas. The staining intensity was reported as the mean of the measured cardiomyocyte gray value minus the background gray value. The background gray value was measured at a cell-free area of the slice. For staining intensity detection, a Zeiss Axioshot microscope coupled to a 3-chip CCD camera was used, and the analysis was performed by using the Optimas 6.01 image analysis program installed on a Pentium PC.

Study Design

We conducted a randomized, double-blind, controlled clinical trial to compare NAC versus placebo on ROS-mediated myocardial injury in the hearts of patients subjected to coronary artery surgery during CPB. Patients and study staff (including end point assessors) were blinded to group assignment. Change (end of CPB – before CPB) in 8-iso-prostaglandin-F2α (nitrotyrosine) density was predefined in the trial protocol as the primary (coprimary) end point for confirmatory statistical analysis (method of a priori–ordered hypotheses).

On the basis of our previous study,a in which we found that the number of 8-iso-prostaglandin-F2α–positive capillaries increased by approximately 100% from before CPB to the end of CPB (from 92 ± 72/mm² before CPB to 209 ± 108/mm² at the end of CPB), we assumed that compared with placebo, NAC would reduce the increase in 8-iso-prostaglandin-F2α–positive capillaries per square millimeter by 50%. Thus a sample size of 20 patients per group seemed adequate to ensure a power of at least 80% at a 2-tailed significance level of 5%.

Statistical Analysis

Continuous variables were summarized as means ± SD. Changes in primary or secondary outcome variables were analyzed for statistical significance at an α level of 5% by using either the 2-tailed Welch modified t test for unpaired samples (to account for unequal variances in groups) or the Student t test for paired samples, where appropriate. Corresponding 95% confidence intervals (CIs) are given to allow assessment of effect sizes for clinical relevance. Statistical analyses were performed with the software package SPSS for Windows, Release 10.0.7. The P values reported for the primary end points need not to be adjusted for multiple testing because a fixed sequence of hypotheses was predefined in the trial protocol.

Results

Immunostaining for 8-iso-prostaglandin-F2α and nitrotyrosine in cardiac myocytes and capillaries is depicted in Figure 2. Compared with conditions before CPB, LV cardiac myocytes of the placebo group demonstrated 8-iso-prostaglandin-F2α and nitrotyrosine formation at the end of CPB. In contrast, cardiac myocytes of the NAC group remained negative for both 8-iso-prostaglandin-F2α and nitrotyrosine at the end of CPB. Because of the small size of the LV biopsy samples, we were able to densitometrically quantitate cardiomyocyte staining for 8-iso-prostaglandin-F2α (before CPB and at the end of CPB) in 19 patients of the placebo group and 17 patients of the NAC group and for nitrotyrosine in 18 patients of the placebo group and 19 patients of the NAC group, respectively. In the placebo group 8-iso-prostaglandin-F2α staining increased from 19.0 ± 5.6 gray units before CPB to 24.0 ± 6.6 gray units at the end of CPB (P < .001; 95% CI for mean change, 3.0-6.9), and nitrotyrosine staining increased from 39.2 ± 8.1 gray units before CPB to 48.3 ± 12.1 gray units at the end of CPB (P < .001; 95% CI for mean change, 5.0-13.4). In the NAC group the change in 8-iso-prostaglandin-F2α staining was not statistically significant (21.7 ± 6.9 gray units before CPB and 19.8 ± 4.1 gray units at the end of CPB; P = .323; 95% CI for mean change, −5.7 to 2.0), and nitrotyrosine staining actually decreased from 45.7 ± 11.4 gray units before CPB to 39.4 ± 9.4 gray units at the end of CPB (P = .012; 95% CI for mean change, −11.2 to −1.6). The changes (from before CPB to the end of CPB) in cardiomyocyte density for 8-iso-prostaglandin-F2α and nitrotyrosine are depicted in Figure 3.

Intraoperative procedures and clinical outcomes were similar between groups; there were no deaths or perioperative myocardial infarctions (Table 2). We did not observe adverse effects attributable to NAC. Post-CPB hemodynamics, including heart rate, vascular pressures, systemic and pulmonary vascular resistance, cardiac index, LV function as measured by FAC, and positive inotropic medication, were similar between groups. The summary results for each study group are displayed in Table 3.
Discussion

Our data show that ROS scavenging with NAC prevents ROS-mediated myocardial stress induced by CPB and cardioplegic arrest because both 8-iso-prostaglandin-F$_2$α and nitrotyrosine formation in LV cardiac myocytes were significantly lower in patients receiving NAC compared with those seen in patients receiving placebo. These data for the first time provide direct evidence that ROS scavenging...
attenuates oxidative stress in hearts of patients subjected to ischemia and reperfusion induced by cardiopulmonary bypass.

Ischemia-reperfusion injury is a multifactorial process involving physical, metabolic, and immunologic components. It affects both cardiac myocytes and coronary endothelial cells and appears to be a major factor contributing to perioperative myocardial damage. Although the pathophysiology of ischemia-reperfusion injury is not yet fully understood, massive ROS production has been identified as an important causative factor mediating the reperfusion injury that characterizes metabolic, structural, and functional myocardial damage associated with myocardial ischemia and reperfusion.1,3 During the ischemic period, adenosine triphosphate consumption leads to accumulation of the purine catabolites hypoxanthine and xanthine, which, on subsequent reperfusion and oxygen influx, are metabolized by xanthine oxidase to produce massive amounts of superoxide and hydrogen peroxide.1 Furthermore, ischemia acts as stimulus for constitutive NO synthase activation,21-23 resulting in increased NO release and subsequent ONOO\textsuperscript{−} production during reperfusion.8 ROS then attack myocardial phospholipids and proteins, leading to formation of 8-iso prostaglandin-F\textsubscript{2α}, the stable end product of arachidonic acid oxidation,6,9 and nitrotyrosine, the stable end product of cell membrane protein–bound tyrosine nitration by ONOO\textsuperscript{−}.10,11 We have previously demonstrated that ischemia and reperfusion induced by cardiopulmonary arrest resulted in both 8-iso prostaglandin-F\textsubscript{2α} and nitrotyrosine formation in LV biopsy specimens of patients subjected to cardiac surgery.8 These data provide a rational basis for clinical investigation of ROS scavengers as adjuncts to myocardial protection strategies.

However, only a few clinical studies have investigated whether ROS scavenging ameliorates myocardial injury associated with CPB and cardioplegic arrest. Even though these studies investigating ROS scavengers, such as deferoxamine,14 NAC,15 or allopurinol either alone7,13 or in combination with vitamins E and C,12 have suggested indirect evidence for beneficial effects, including reduced neutrophil oxidative burst response14,15 and improved patient outcome,7,12,13 none of these studies directly investigated the effect of ROS scavenging on myocardial tissue alterations. In the present study the antioxidant and ROS scavenger NAC completely prevented myocardial 8-iso prostaglandin-F\textsubscript{2α} formation induced by cardiopulmonary arrest and subsequent reperfusion. To our knowledge, this is the first study providing direct evidence that ROS scavenging attenuates oxidative stress in the hearts of patients subjected to cardiopulmonary bypass. Interestingly, we found that cardiac myocyte nitrotyrosine density actually decreased from before CPB to the end of CPB in the NAC group. This indicates that at the time of the first LV biopsy, some nitrotyrosine must have already been present, probably as a result of non-specific inflammatory ONOO\textsuperscript{−} production induced by stress, the surgical trauma per se, or both.1 Thus to yield the full potential benefit of its ROS-scavenging properties, NAC application should probably begin before anesthesia induction.

The functional correlate of ROS-mediated tissue injury is myocardial stunning.2,3 Recent experimental studies have shown that 8-iso prostaglandin-F\textsubscript{2α} resulted in coronary vasoconstriction associated with lactate production and contractile dysfunction.24 This coronary vasoconstriction persisted even after removal of the ROS stimulus and might thus contribute to the no-reflow phenomenon during reperfusion.25 In addition, ONOO\textsuperscript{−} production caused by increased NO release has been demonstrated to be associated with irreversible cardiac function impairment26 and higher mortality after coronary occlusion27 and might be involved in cardiac apoptosis initiation.11 Finally, Gupta and Okada28 recently demonstrated in isolated rat hearts that ROS mediate LV dysfunction through ONOO\textsuperscript{−} production that can be attenuated by NO synthase inhibition. In the present study global LV ejection fraction, as measured on the basis of FAC, cardiac index, and clinical patient outcome, was similar between the NAC and placebo groups, and thus the functional and clinical relevance of ROS scavenging remains to be evaluated. However, cardiac performance directly determined after CPB might not reflect potential long-term effects attributable to ROS, determination of which is beyond the scope of the present acute study. Considering the role of ROS in metabolic and structural myocardial alterations, as well as cardiac apoptosis induction potentially impairing cardiac performance, attenuation

Figure 3. Change from before CPB to the end of CPB in cardiomyocyte density for 8-iso-prostaglandin-F\textsubscript{2α} and nitrotyrosine in both groups. Data are presented as means ± SD (for 8-iso prostaglandin-F\textsubscript{2α}, n = 19 in the placebo group and n = 17 in the NAC group; for nitrotyrosine, n = 18 in the placebo group and n = 19 in the NAC group).
of ROS-mediated myocardial tissue alterations by NAC has to be regarded as a cardioprotective measure.

In conclusion, the data of the present study for the first time provide direct evidence that ROS scavenging with NAC attenuates oxidative stress in the hearts of patients subjected to CPB and cardioplegia. Because we did not observe adverse effects attributable to NAC, our data indicate that routine NAC administration might be a useful tool to attenuate ROS-mediated myocardial stress in patients undergoing cardiac surgery.

References


