The antioxidant N-acetylcysteine preserves myocardial function and diminishes oxidative stress after cardioplegic arrest

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Objective: Oxidative stress contributes to myocardial ischemia-reperfusion injury. We hypothesized that administration of the antioxidant N-acetylcysteine would have beneficial effects on myocardial function after cardiopulmonary bypass and cardioplegic arrest.

Methods: Anesthetized dogs (n = 18) were instrumented with myocardial ultrasonic crystals and a left ventricular micromanometer. Systolic function was measured by preload recruitable stroke work. Myocardial tissue water was determined by microgravimetry. Treated animals received 100 mg · kg⁻¹ N-acetylcysteine 10 minutes before initiation of cardiopulmonary bypass followed by 20 mg · kg⁻¹ · h⁻¹ continuous infusion until 1 hour after cardiopulmonary bypass. After baseline, cardiopulmonary bypass and 2-hour crystalloid cardioplegic arrest was initiated, then reperfusion/rewarming for 40 minutes and separation from cardiopulmonary bypass. Myocardial function parameters and myocardial tissue water were measured at 30, 60, and 120 minutes after cardiopulmonary bypass. Oxidative stress was measured by 8-isoprostane concentrations in the coronary sinus plasma.

Results: Preload recruitable stroke work did not decrease from baseline in the N-acetylcysteine group and was significantly greater in N-acetylcysteine group compared with controls at 30 (104% ± 9% vs 80% ± 4%; P < .05) and 120 minutes (98% ± 7% vs 79% ± 4%; P < .05) after cardiopulmonary bypass. Concentrations of 8-isoprostane in the coronary sinus plasma of the control dogs were significantly higher 30 minutes after cardiopulmonary bypass compared with baseline but were unchanged in the N-acetylcysteine group. Myocardial edema resolution was significantly greater in the N-acetylcysteine group at 30 minutes after cardiopulmonary bypass compared with control (−2.5% ± 0.7% vs −0.3% ± 0.5% myocardial tissue water; P < .05).

Conclusions: Administration of the antioxidant N-acetylcysteine preserves systolic function and enhances myocardial edema resolution after cardiopulmonary bypass/cardioplegic arrest. Furthermore, oxidative stress was significantly reduced in the treated animals. Therefore, our findings support the hypothesis that oxidative stress is the main cause for myocardial dysfunction after ischemia-reperfusion.

Myocardial protection during cardiopulmonary bypass (CPB) and cardioplegic arrest (CA) has continued to be refined since its introduction.1 Despite numerous advances the heart may still exhibit evidence of ischemia-reperfusion injury, such as arrhythmias, microvascular damage, edema, myocardial stunning, and cell death.2,3 A mechanism by which ischemia-reperfusion may produce these findings is the generation of reactive oxygen species...
(ROS), which has been shown to form during the early phase of reperfusion.\textsuperscript{4,5} The alterations in membrane permeability, configuration, and cellular proteins due to ROS have been suggested as a main cause for ischemia-reperfusion injury.\textsuperscript{6} To eliminate or at least diminish the effects of ROS associated with CPB/CA, several studies have been performed using antioxidants.\textsuperscript{7,12} The results are inconclusive, most likely due to factors such as species differences, different experimental methods, and different types of antioxidants.

After examination of the literature we chose N-acetyl-cysteine (NAC) as a nonspecific antioxidant for our study. NAC interacts with oxygen radicals and results in NAC-disulfide as a main end product.\textsuperscript{13} In addition to its function as oxygen radical scavenger, NAC also inhibits oxygen radical generation by polymorphonuclear leukocytes in vitro and in vivo.\textsuperscript{14,15} The effect of NAC on reperfusion injury in a number of experiments including CPB has been investigated. Andersen and colleagues\textsuperscript{7} showed that NAC significantly diminished neutrophil oxidative burst response throughout CPB. Another study reported that NAC could prevent secondary lung reperfusion injury after liver ischemia-reperfusion.\textsuperscript{16} Furthermore, NAC prevented radiographic contrast-agent-induced reductions in renal function due to ROS.\textsuperscript{17} Thus, NAC appears to be a promising substance for reduction of oxidative stress related to CPB/CA.

The purpose of our study was to evaluate the effect of NAC administration on ROS-mediated ischemia-reperfusion injury associated with CPB/CA. Therefore, we chose to measure preload recruitable stroke work (PRSW) and myocardial tissue water (MWC) as depressed left ventricular (LV) function and myocardial edema are major components of ischemia-reperfusion injury.\textsuperscript{3} We hypothesized that NAC would preserve myocardial function after CA and CPB by oxidative stress reduction.

\section*{Methods}

\subsection*{Animal Preparation}

All procedures were approved by the University of Texas Animal Welfare Committee. Eighteen conditioned mongrel dogs of either sex (29.2 ± 3.4 kg) were anesthetized with 25 mg/kg thiopental sodium intravenously and endotracheally intubated. The lungs were mechanically ventilated with 100% oxygen using a volume-cycled ventilator (Servo ventilator 900C, Siemens-Elema, Solna, Sweden). Anesthesia was maintained with an intravenous infusion of 1% thiopental sodium in lactated Ringer’s solution.

Fluid-filled catheters were inserted in the left femoral artery and vein for mean arterial pressure monitoring, arterial blood sampling, and fluid administration. A Swan-Ganz catheter (Edwards LifeSciences, Irvine, Calif) was inserted via the right jugular vein into the pulmonary artery for central venous pressure, pulmonary artery pressure (PAP), pulmonary artery wedge pressure (PCWP), and cardiac output (CO) determinations. The pressure-monitoring catheters were connected to calibrated pressure transducers (Isotec, Healthdyne Cardiovascular, Irvine, Calif) and data were recorded on a computer (MacLab, WorldPrecision Instruments, Sarasota, Fla). The right femoral artery was exposed for subsequent CPB cannulation. After a median sternotomy and peri-cardiotomy, a 7F catheter was placed into the coronary sinus for coronary sinus blood sampling. Subsequently we placed sonomicrometry crystals (10 MHz, Sonometrics, London, Ontario, Canada) in the subendocardium at midventricular level across the septum/free wall axis of the left ventricle. A micromanometer-tipped pressure transducer (Millar Instruments Inc, Houston, Tex) was introduced in the LV cavity through the apex. We placed a snare around the inferior vena cava for cardiac preload manipulation.

\subsection*{CPB and Cardioplegic Arrest}

After preparation, heparin (250 IU/kg) was given intravenously for systemic anticoagulation. Additional doses of 75 IU/kg heparin were administered every 60 minutes throughout the experiment. A 16F arterial perfusion cannula was introduced into the prepared right femoral artery. A 2-stage (34F/38F) venous cannula was placed into right atrium/inferior vena cava. The LV chamber was vented with a 12F catheter inserted via the left atrium. Three roller pumps were used for extracorporeal circulation, left ventricular drainage, and suction (Sarns Inc, Ann Arbor, Mich). The extracorporeal circuit and the membrane oxygenator (Cobe Cardiovascular Inc, Arvada, Colo) were primed with 1000 mL of lactated Ringer’s solution and 1000 IU of heparin. In 9 dogs (group NAC) 10 minutes before initiation of CPB, NAC (Sigma Chemical Co, St Louis, Mo) was dissolved in 0.9% NaCL and administered intravenously as a bolus of 100 mg/kg (50 mg/mL) followed by an infusion of 20 mg/kg per hour (50 mg/mL).\textsuperscript{7,16} In 9 dogs that served as controls (group CON), the same fluid amount without NAC was applied. The body temperature was cooled to 28°C. The aorta was crossclamped, and 500 mL iced (approximately 4°C) crystalloid cardioplegic solution (Plegisol; Abbott Laboratories, Chicago, Ill) was infused into the aortic root at 80 mm Hg. An additional 100 mL of cardioplegic solution was given after 60 minutes of cardiac arrest or if electrical or mechanical activity of the heart occurred. There was no difference concerning the amount of administered cardioplegic solution between the groups. Whole-body hypothermia at 28°C was maintained for 105 minutes of aortic crossclamping, followed by rewarming to 37°C. After 120 minutes of CA, the aortic crossclamp was removed, and the heart was reperfused on normothermic CPB for 40 minutes. The dogs were weaned from CPB and all cannulas were removed. We kept CPB flow between 40 and 60 mL · kg\textsuperscript{-1} · min\textsuperscript{-1} to maintain a systemic perfusion pressure of 40 to 70 mm Hg. No inotropic or antiarrhythmic agents were required for separation from CPB or in the post-CPB period. In the NAC group, NAC infusion was continued until 60 minutes after CPB.

\subsection*{Interstitial Myocardial Edema}

Myocardial water content was measured from endomyocardial biopsies using microgravimetric technique as previously described.\textsuperscript{18} In brief, a linear density gradient was prepared in a gradient former from 2 different mixtures of kerosene and bromo-
benzene, which were adjusted to a specific gravity of 0.990 and 1.080, respectively. The gradient was calibrated with various K₂SO₄ solutions with known specific gravities and by recording the equilibration depth of 10 μL drops of the various solutions. Linearity of the gradient was confirmed by linear-square regression analysis after plotting the equilibration depths versus specific gravity. The mean correlation coefficient was 0.96 ± 0.008 (n = 18). To determine the specific gravity of myocardium, we introduced an endoluminal biopsy forceps transapically into the left ventricle and collected myocardial samples. These samples were placed gently into the density column and the equilibration depth was recorded after 1 minute. MWC can be calculated using the equation: MWC = (1 − [(SGₘy/o − 1)/(1 − 1/SGₙ)d]) × 100 (%), where SGₘy/o and SGₙd are the specific gravities of the myocardial tissue and dry myocardium, respectively. On conclusion of the experiment, the dog was killed with an intravenous overdose of thiopental sodium (Pentothal) and saturated potassium chloride. After a last myocardial tissue density measurement, the heart was rapidly excised. The right and left ventricle were then weighed and dried at 60°C in an oven to a constant weight. We assume that SGₙd did not change over the experimental period. Edema resolution was defined as decrease in MWC 2 hours after CA.

8-Isoprostane Determination in Coronary Sinus Plasma
To evaluate the ROS-mediated tissue injuries we used an assay of 8-iso-prostaglandin-F₂α (8-isoprostane); 8-isoprostane is the stable end product of arachidonic acid oxidation generated by ROS injury to membrane phospholipids and has been used as an indicator for free radical-mediated injury in hearts of patients subjected to cardioplegia. The concentration of 8-isoprostane was measured in coronary sinus plasma using a kit (Cayman Chemical Company, Ann Arbor, Mich).

Experimental Protocol
After instrumentation, we recorded baseline measurements of CO, mean arterial pressure, PAP, PCWP, central venous pressure, and LV pressure-volume loops. Two myocardial samples for MWC determination were collected. We placed the dog on CPB and initiated cardiac arrest as described above. We measured all variables at 30, 60, 90, and 120 minutes during cardiac arrest. At 30, 60, and 120 minutes after separation from CPB we repeated all measurements. Myocardial samples were taken parallel to all measurements.

Statistical Analysis
We examined the time courses of each measured parameter using analysis of variance for repeated measures and the Student t test for comparisons between the groups. Post hoc comparisons were performed using the Tukey test.

Results
Hemodynamic Parameters
Heart rate (HR) was significantly increased in the CON group in the post-CPB period compared with baseline, whereas HR remained stable in the NAC group. Mean arterial pressure was significantly decreased in both groups after weaning from CPB compared with baseline values, but remained stable thereafter. Cardiac index did not change in either group (see Table 1).

Contractile Function
After weaning from CPB, the PRSW values in the NAC group did not change compared with baseline. In the control group, PRSW was significantly decreased compared with baseline and was significantly lower at 30 and 120 minutes after CPB compared with the NAC group (Table 1).

Isovolumic Relaxation
In both groups τ was increased with myocardial edema formation after CPB but was only significant in the NAC group at 60 minutes after CPB; rate of pressure rise (−dP/dtmax) was significantly decreased in both groups after weaning from bypass compared with baseline values and did not recover with edema resolution within 120 minutes after CPB (Table 1).

Myocardial Water Content
In both groups MWC increased after initiation of cardiac arrest by Plegisol administration (Table 1). After weaning from CPB, myocardial edema resolved but was still significant 120 minutes after CPB compared with baseline in either group. However, edema resolution 30 minutes as measured by decrease in MWC after weaning from CPB was significantly higher in the NAC group compared with the CON group (Figure 1).

8-Isoprostane Concentrations in the Coronary Sinus Plasma
The concentration of 8-isoprostane in the coronary sinus plasma of the control dogs was significantly higher at 30 minutes after CPB compared with baseline (223% ± 97%; P < .05) and with the NAC group. Values of 8-isoprostane in the NAC group were not significantly changed compared with baseline levels (89% ± 50%; see Figure 2).

Discussion
Our data show that NAC administration during CPB and CA preserved LV function and improved myocardial edema resolution in the early phase after weaning from CPB.

LV Function
We believe that the preserved LV systolic function in the NAC group is due to the ROS reduction during CPB and especially during the early reperfusion period. Increased generation of ROS results in alterations of membrane permeability, configuration, and cellular proteins, which in turn are responsible for LV systolic dysfunction as part of ischemia-reperfusion damages to the heart. A reduction of the ROS generation during CPB and especially during reperfu-
sion is therefore most likely the explanation for the preserved LV systolic function in the NAC group.

Although oxidative stress is the main cause for ischemia-reperfusion injury, a beneficial effect of antioxidants on cardiac ischemia-reperfusion injury could not be shown for all agents used in previous studies. These inconsistent findings could be due to study differences. We chose to administer NAC over the whole period of CPB until 60 minutes after weaning from CPB because CPB itself is associated with oxidative stress. Compared with other studies, the protective effect covered the whole CPB and reperfusion period, suggesting that an extended protection against oxygen radicals is more efficient in reducing oxidative stress and preserving cardiac function.

The type of antioxidant has also been suggested to be responsible for controversial effects on myocardial performance after ischemia-reperfusion.

Some of the beneficial effects of NAC have been attributed to its chemical structure that includes a thiol-containing compound. In studies with CPB and ischemic preconditioning, thiol-containing compounds have been shown to be beneficial and have been suggested as potent defense systems against ischemia-reperfusion–related oxidative stress.

We know that the NAC dose used in this study was sufficient, as evidently 8-isoprostane concentrations were unchanged in the treated dogs compared with baseline, which is consistent with the findings of Andersen and colleagues.

**Myocardial Edema Resolution**

The significantly higher decrease in MWC during the first 30 minutes after CPB and therefore improved edema resolution in the NAC group could be due to the following reasons. Allen and coworkers showed that augmented

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<tr>
<th>TABLE 1. Hemodynamic parameters, left ventricular diastolic function, and myocardial water content</th>
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<tr>
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<tr>
<td><strong>HR (bpm)</strong></td>
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<tr>
<td>Baseline                      30 minutes post-CPB                      60 minutes post-CPB                      120 minutes post-CPB</td>
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<tr>
<td><strong>CON</strong>                  126 ± 14                                        144 ± 13*                                          145 ± 12*                                          135 ± 14*</td>
</tr>
<tr>
<td><strong>NAC</strong>                  133 ± 11                                        138 ± 15                                            138 ± 15                                            126 ± 15</td>
</tr>
<tr>
<td><strong>MAP (mm Hg)</strong></td>
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<tr>
<td><strong>CON</strong>                  122 ± 12                                        82 ± 13*                                          82 ± 9*                                          96 ± 17*</td>
</tr>
<tr>
<td><strong>NAC</strong>                  118 ± 22                                        89 ± 9*                                          86 ± 10*                                          91 ± 11*</td>
</tr>
<tr>
<td><strong>CVP (mm Hg)</strong></td>
</tr>
<tr>
<td><strong>CON</strong>                  9 ± 3                                           10 ± 2                                           9 ± 2                                           9 ± 2</td>
</tr>
<tr>
<td><strong>NAC</strong>                  7 ± 1                                           8 ± 1*                                          8 ± 1*</td>
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<tr>
<td><strong>PAP (mm Hg)</strong></td>
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<tr>
<td><strong>CON</strong>                  19 ± 4                                         21 ± 4                                           23 ± 4*                                          23 ± 6*</td>
</tr>
<tr>
<td><strong>NAC</strong>                  16 ± 3                                         21 ± 2*                                          21 ± 1*                                          21 ± 2*</td>
</tr>
<tr>
<td><strong>PCWP (mm Hg)</strong></td>
</tr>
<tr>
<td><strong>CON</strong>                  10 ± 3                                         13 ± 6*                                          12 ± 4*                                          14 ± 4*</td>
</tr>
<tr>
<td><strong>NAC</strong>                  9 ± 3                                          11 ± 2                                           10 ± 2                                           10 ± 4*</td>
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<tr>
<td><strong>Cardiac index (L/min per m²)</strong></td>
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<tr>
<td><strong>CON</strong>                  3.08 ± 0.87                                      3.19 ± 1.63                                      3.35 ± 2.34                                      2.87 ± 1.45</td>
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<tr>
<td><strong>NAC</strong>                  3.26 ± 1.53                                      3.05 ± 1.39                                      2.87 ± 1.88                                      2.25 ± 1.17</td>
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<tr>
<td><strong>dP/dt max (mm Hg/s)</strong></td>
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<tr>
<td><strong>CON</strong>                  −1916 ± 432                                     −1373 ± 520*                                     −1320 ± 508*                                     −1235 ± 269*</td>
</tr>
<tr>
<td><strong>NAC</strong>                  −1886 ± 433                                     −1168 ± 226*                                     −1185 ± 302*                                     −1099 ± 456*</td>
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<tr>
<td><strong>MWC (%)</strong></td>
</tr>
<tr>
<td><strong>CON</strong>                  79.35 ± 2.25                                     82.03 ± 1.93*                                    81.14 ± 3.25*                                    80.23 ± 2.82*</td>
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<tr>
<td><strong>NAC</strong>                  77.57 ± 0.69                                     81.19 ± 1.41*                                    80.79 ± 1.40*                                    80.37 ± 1.18*</td>
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<tr>
<td><strong>PRSW (% bl) (mm Hg)</strong></td>
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<tr>
<td><strong>CON</strong>                  45 ± 13                                        47 ± 15                                          49 ± 13                                          41 ± 12</td>
</tr>
<tr>
<td><strong>NAC</strong>                  40 ± 9                                          48 ± 20                                         51 ± 20*                                          45 ± 15</td>
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Values are mean ± standard deviation. HR, Heart rate; CI, cardiac index; MAP, mean arterial pressure; PAP, pulmonary artery pressure; CVP, central venous pressure; PCWP, pulmonary capillary wedge pressure; r, isovolumic relaxation constant; −dP/dt max, pressure at negative dP/dt max; MWC, myocardial water content; PRSW, preload recruitable stroke work; CPB, cardiopulmonary bypass.

*P < .05 for difference from baseline period.
†P < .05 for difference between the groups.
contractility hastens myocardial edema resolution. The preserved contractility after weaning from CPB in the NAC group could explain the better edema resolution compared with control.

Differences in edema resolution between the groups could also be explained by impaired myocardial lymphatic function due to increased ROS concentration during the early phase of reperfusion in the control group. Oxidative stress has been shown to impair lymphatic function.24,25 The changes in edema resolution between the groups could only be found within 30 minutes after CPB. In this period the oxidative stress as measured by 8-isoprostane concentrations in the coronary sinus plasma was still higher in the CON group compared with NAC. Thus, impaired lymphatic function due to increased ROS concentration might have contributed to a slower edema resolution in the CON group.

Myocardial edema was not completely resolved at the end of the experiment in either group and therefore explains the impaired diastolic function as measured by \(\tau\) and \(-dP/dt_{\text{max}}\).

Additional Effects of NAC
According to Andrews and colleagues,26 NAC improves coronary vascular function as measured by coronary artery flow. We did not investigate possible differences in coronary vascular flow between the NAC and CON groups. As oxidative stress is the main cause for ischemia-reperfusion injury, we believe that NAC functioned mainly as antioxidant. However, an improved vascular function in the NAC group may have contributed to preserve myocardial function.

Clinical Implications
Although it is uncertain whether our findings can be extrapolated to clinical situations, we believe that the animal data is strong enough to warrant the consideration of a clinical trial because beneficial effects of NAC on myocardial function after CPB and CA have been shown in other species.3 Andersen and coworkers7 showed that NAC in the dosage we used in our study reduced the neutrophil oxidative response during CPB in patients undergoing coronary artery bypass surgery without adverse effects. In our study we also did not see any adverse effects. Whether this concentration is also sufficient to depress the oxidative burst at the beginning of reperfusion after CA in patients has to be evaluated. However, even higher NAC concentrations could be used with little risk of adverse effects27,28. Furthermore, NAC in equal or even higher doses has been successfully used in clinical studies for the treatment of acute myocardial infarction.29,30

Our findings and the clinical studies using NAC in the context of local ischemia-reperfusion demonstrate that NAC administration improves myocardial performance and significantly diminishes oxidative stress after cardiac surgery with CPB and CA. Therefore, we believe that clinical studies using NAC in the context of CPB and CA are warranted.

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References
4. Bolli R. Causative role of oxyradicals in myocardial stunning: a proven hypothesis. A brief review of the evidence demonstrating a