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N-Acetylcysteine for Preventing Pump-Induced Oxidoinflammatory Response During Cardiopulmonary Bypass

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Abstract

Purpose. To investigate the effect of *N*-acetylcysteine on preventing pump-induced oxidoinflammatory response during cardiopulmonary bypass (CPB).

Methods. Forty patients undergoing coronary artery bypass grafting (CABG) were randomly divided into a study group ($n = 20$), given 50 mg kg^{-1} *N*-acetylcysteine intravenously for 3 days, and a control group ($n = 20$) given saline. Serum samples were collected for measurement of myeloperoxidase (MPO), malondialdehyde (MDA), interleukin-6, α_1-acid glycoprotein (AAGP), and C-reactive protein (CRP) during surgery and postoperatively.

Results. The MPO and MDA values showed a similar pattern during and after CPB in the study group, with significantly less variance than in the control group. Interleukin-6 showed similar patterns in the two groups, but the data from 30 min after the start of CPB and from 6 h post-CPB were significantly different. The AAGP and CRP values were both elevated during CPB in the two groups without a significant difference, but 6 and 24 h post-CPB, the values were significantly higher in the control group than in the study group.

Conclusions. *N*-Acetylcysteine decreased pump-induced oxidoinflammatory response during CPB, suggesting that it could be a novel therapy for assisting in the prevention of CBP-induced oxidoinflammatory damage.

Key words *N*-Acetylcysteine · Cardiopulmonary bypass · Interleukin-6 · Lipid peroxidation · Myeloperoxidase · Acute phase reactant

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Introduction

Systemic inflammatory response to cardiopulmonary bypass (CPB) is attributed to contact of the blood components with the artificial surface of the bypass circuit, ischemia/reperfusion (I/R) injury, endotoxemia, and operative trauma.[1,2] Activation of the inflammatory pathways during CPB induces a systemic inflammatory response by triggering the production and release of various inflammatory mediators, leading to leukocyte activation as well as neutrophil and endothelial cell adhesion molucule expression.[3–5] The release of reactive oxygen or nitrogen species and proteases is believed to be primarily responsible for the neutrophil-related damage to the endothelial integrity,[6] while the activation of leukocytes and their sequestration into target organs is considered to be the main cause of capillary leakage and organ dysfunction.[7–9]

Several attempts have been made to prevent CPB-related organ failure in recent years. The techniques used include leukocyte filtering, inhibitor release of various endogenous inflammatory mediators, receptor antagonist of these mediators, immunomodulation, and monoclonal antibodies to prevent pump-induced damage.[1,2] Despite some technological improvements in CPB equipment, no single anti-inflammatory or immunological therapy has been accepted for routine use in clinical practice. However, one of the most popular methods of inhibiting oxidant-mediated injury involves the use of glutathione-modulating agents such as thiol or sulfhydryl compounds, among which *N*-acetylcysteine (NAC) is probably on of the most widely investigated agents, because it serves as a precursor of glutathione and acts as a direct scavenging agent.[10] In addition to its antioxidant properties, NAC elicits other beneficial effects through anti-inflammatory action, by suppressing cytokine expression/release, and inhibiting adhesion molecule expression and nuclear factor kappa B.[11–13] *N*-Acetylcysteine pretreatment could be a

promising approach to attenuate the negative effects of CBP on postoperative organ function. However, the clinical role of NAC in alleviating CBP-induced inflammatory response has yet to be elucidated. The present study was conducted to investigate the effects of clinical NAC pretreatment on neutrophil activation, lipid peroxidation, and the acute-phase reactants of patients undergoing CPB, by measuring myeloperoxidase (MPO) activity, interleukin-6 (IL-6), malondialdehyde (MDA), C-reactive protein (CRP), and α_1-acid glycoprotein (AAGP) levels.

Patients and Methods

Study Protocol

This study was approved by the ethics committee of our institution and informed consent was obtained from all patients. Forty patients with stable angina pectoris who underwent coronary artery bypass grafting (CABG) were randomly divided into two groups. The study group ($n = 20$) received 50 mg kg^{-1} NAC intravenously per day, given as 25 mg kg^{-1} twice in 24 h, and the control group ($n = 20$) received 0.5 cc kg^{-1} saline twice in 24 h for 3 days before CABG. Patients with poor ventricular function (ejection fraction <30%) or diabetes mellitus, and those requiring emergency surgery were excluded. Any patients exhibiting remarkably abnormal pulmonary, endocrine, metabolic, or neurologic pathology were also excluded. All cardiac medications were continued until the day before surgery. After the completion of CABG, patients were transferred to the intensive care unit. Postoperative care was standardized for all patients, and extubation was done as early as possible.

Cardiopulmonary Bypass

All patients were given 1 mg alprazolam orally (Xanax, Eczacıbası, Turkey) for sedation the night before surgery. Following neuroleptic anesthesia, a standard median sternotomy was performed, and two-stage venous and aortic cannulation and continuous-flow CPB with moderate hypothermia was initiated. After cross-clamping the aorta, cold crystalloid cardioplegic solution (Plegisol, Abbott, Abbott Park, IL, USA) was administered at an initial dose of 15 cc kg^{-1} and blood cardioplegia was given every 20 min. All of the patients received warm blood cardioplegia just before the aortic cross-clamp was removed, and the proximal anastomoses were performed after the application of a side clamp.

Blood Sampling

Arterial blood samples were collected after the induction of anesthesia (t_1), then 5 min (t_2) and 30 min (t_3) after CPB, at the end of surgery (t_4), and 6 h (t_5) and 24 h (t_6) after the cessation of CPB.

Biochemical Assay

Measurement of Myeloperoxidase (MPO)

The level of sera MPO activity is directly related to the fact that it reduces o-dianisidine. Therefore, the reduction in o-dianisidine was measured by spectrophotometry at the wavelength of 410 nm for estimation of MPO levels.[14]

Measurement of Malondialdehyde (MDA)

The MDA level was measured as an index of lipid peroxidation, by the thiobarbituric acid reaction according to the methods of Yagi.[15] The principle of this method depends on assessment of the pink color produced by the interaction of the barbituric acid with MDA elaborated as a result of lipid peroxidation. The color reactant 1,1,3,3-tetraethoxypropane was used as the primary standard.[15,16]

Measurement of Interleukin-6 (IL-6)

Interleukin-6 levels were measured by enzyme-linked immunosorbent assay using a commercial kit (Roche Diagnostic, Mannheim, Germany).

Measurment of α_1-Acid Glycoprotein (AAGP) and C-Reactive Protein (CRP)

Acute-phase protein levels were measured by immunoturbidometrical methods using Cobas Integra 700 (Roche Diagnostic).

Statistical Analysis

Statistical analysis was performed with SPSS statistical software (SPSS version 9.0, 1989–1999). Categorical data were analyzed using the χ^2-test or Fisher's exact test where appropriate. Student's *t*-test was used to compare the differences in biochemical results between the two groups. Results are expressed as mean ± SD. A *P* value of less than 0.05 was accepted as significant.

Results

The demographic features, clinical characteristics, and intraoperative data did not differ significantly between the two groups (Table 1). We did not observe any significant side effects of NAC during the preoperative, perioperative, and postoperative periods. None of the

Table 1. Demographic, clinical, and surgical data of the control and study groups

	Control group (n = 20)	Study group (n = 20)
Age (years)	64 ± 6	66 ± 4
Sex (M/F)	14/6	15/5
BMI		
≤25	13 (65%)	14 (70%)
>25	7 (35%)	6 (30%)
Preoperative treatment		
Digoxin	2	2
Diuretic	3	2
Nitrate	11	9
Calcium-channel blockers	5	4
Beta-blockers	6	5
CPB duration (min)	108 ± 14	112 ± 16
Duration of aortic cross-clamping (min)	65 ± 9	60 ± 11
Lowest temperature (°C)	28.0 ± 0	28.0 ± 0
Hematocrit before induction	0.41 ± 0.3	0.43 ± 0.4
Min. hematocrit during CPB	0.26 ± 0.2	0.27 ± 0.3
Surgical data		
Saphenous vein grafts	2.3 ± 0.4	2.5 ± 0.3
Internal mammary artery	18	17
Inotropic support after CPB	4	3
Perioperative MI	1	0

BMI, body mass index; CPB, cardiopulmonary bypass; MI, myocardial infarction

patients required reoperation or prolonged intensive care. All patients were transferred from the intensive care unit to a hospital ward by postoperative day 2 at the latest.

The MPO, MDA, and IL-6 values started to increase about 5 min after the commencement of CPB and remained high until 6 h after the cessation of CPB. The levels were low again by 24 h after the cessation of CPB.

The MPO levels were significantly lower in the study group than in the control group. By the end of surgery and 6 h after the cessation of CPB, the MPO levels were significantly higher in the control group than in the study group. The MPO levels had not returned to the preoperative levels by 24 h after the cessation of CPB in the control group (Fig. 1; $P = 0.592$ at t_1, $P = 0.015$ at t_2, $P = 0.010$ at t_3, $P = 0.000$ at t_4, $P = 0.000$ at t_5, $P = 0.000$ at t_6).

The IL-6 levels showed a similar pattern. The study group patients had significantly lower levels of IL-6 than the control group patients at the time periods of t_3, t_4, and t_5. However, by 24 h after the cessation CPB, the IL-6 levels were similar in both groups (Fig. 2; $P = 0.015$ at t_1, $P = 0.609$ at t_2, $P = 0.018$ at t_3, $P = 0.000$ at t_4, $P = 0.000$ at t_5, $P = 0.092$ at t_6).

The MDA levels were also increased during CPB, peaking 6 h after the cessation of CPB, then starting to fall thereafter. *N*-Acetylcysteine pretreatment also seemed to prevent MDA formation when compared with the control group values (Fig. 3; $P = 0.675$ at t_1, $P = 0.049$ at t_2, $P = 0.000$ at t_3, $P = 0.000$ at t_4, $P = 0.000$ at t_5, $P = 0.000$ at t_6).

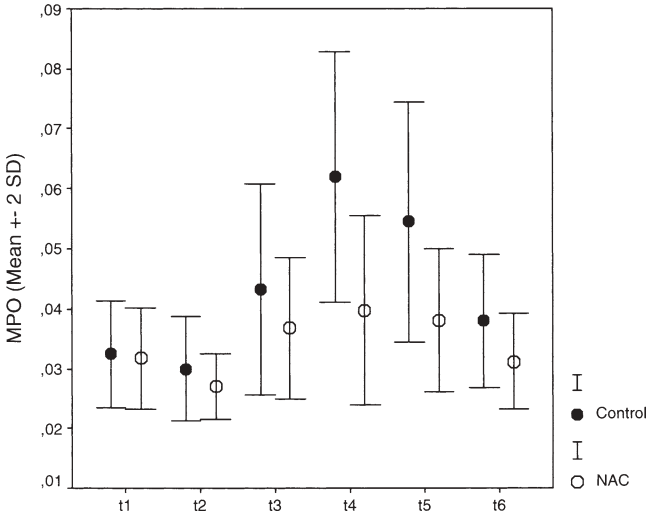

Fig. 1. Myeloperoxidase (*MPO*) values during the operative and postoperative periods. t_1, after induction of anesthesia; t_2, 5 min after cardiopulmonary bypass (CPB); t_3, 30 min after CPB; t_4, at the end of surgery; t_5, 6 h after the cessation of CPB; t_6, 24 h after the cessation of CPB. MPO values are given as U (mg protein)$^{-1}$ h^{-1}

The AAGP and CRP values showed a similar pattern, increasing soon after CPB without a significant difference between the control and study

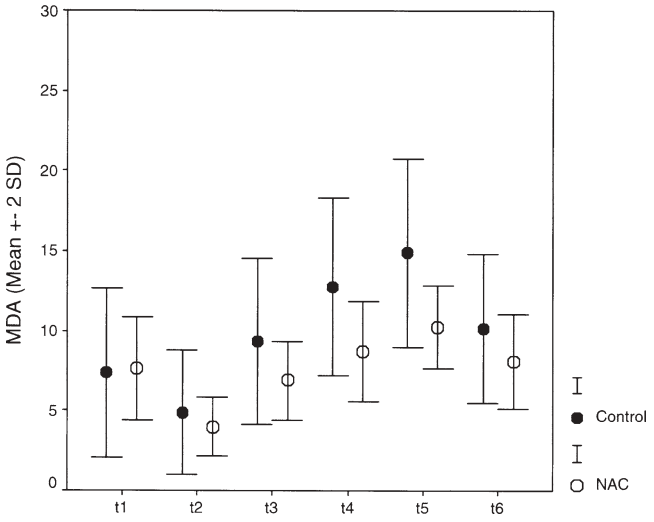

Fig. 2. Malondialdehyde (*MDA*) values during the operative and postoperative periods. t_1, after induction of anesthesia; t_2, 5 min after CPB; t_3, 30 min after CPB; t_4, at the end of surgery; t_5, 6 h after the cessation of CPB; t_6, 24 h after the cessation of CPB. MDA values are given as nmol ml^{-1}

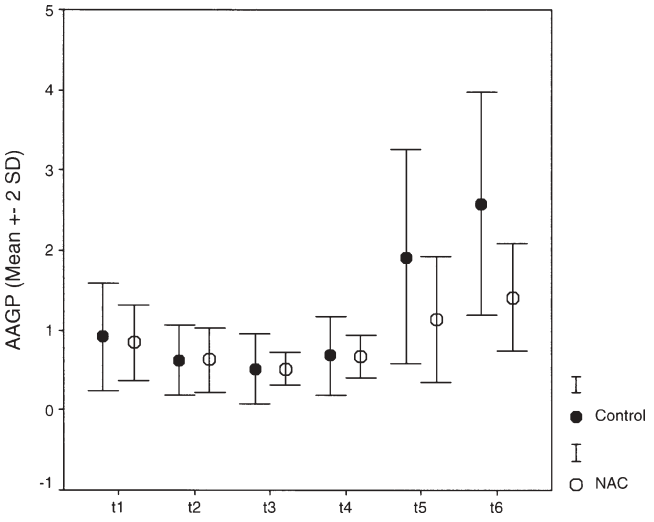

Fig. 4. α_1-Acid glycoprotein (*AAGP*) values during the operative and postoperative periods. t_1, after induction of anesthesia; t_2, 5 min after CPB; t_3, 30 min after CPB; t_4, at the end of surgery; t_5; 6 h after the cessation of CPB. t_6, 24 h after the cessation of CPB. AAGP values are given as g l^{-1}

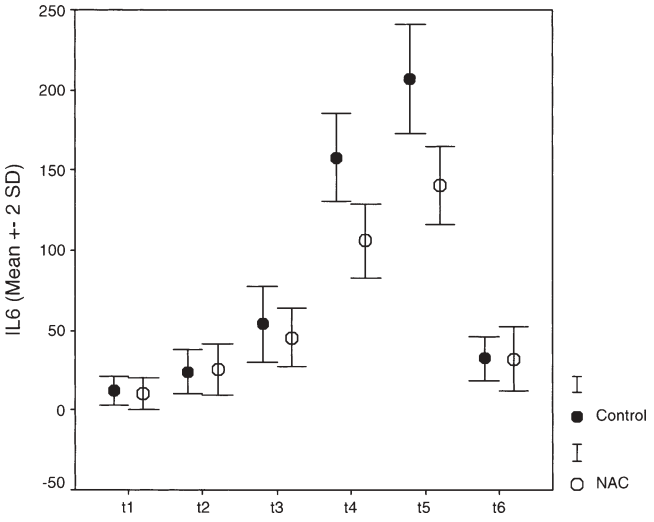

Fig. 3. Interleukin 6 (*IL-6*) values during the operative and postoperative periods. t_1, after induction of anesthesia; t_2, 5 min after CPB; t_3, 30 min after CPB; t_4, at the end of surgery; t_5, 6 h after the cessation of CPB; t_6, 24 after the cessation of CPB. IL-6 values are given as ng l^{-1}

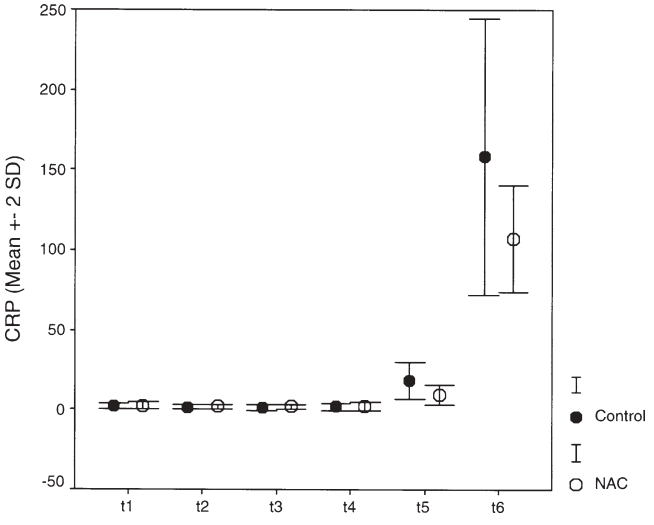

Fig. 5. C-Reactive protein (*CRP*) values during the operative and postoperative periods. t_1, after induction of anesthesia; t_2, 5 min after CPB; t_3, 30 min after CPB; t_4, at the end of surgery; t_5, 6 h after the cessation of CPB; t_6, 24 h after the cessation of CPB. CRP values are given as mg l^{-1}

groups. However, by 6 and 24 h post-CPB, both these acute-phase protein levels were significantly higher in the control group than the study group (Fig. 4; $P = 0.498$ at t_1, $P = 0.944$ at t_2, $P = 0.941$ at t_3, $P = 0.914$ at t_4, $P = 0.000$ at t_5, $P = 0.000$ at t_6. Fig. 5; $P = 0.744$ at t_1, $P = 0.477$ at t_2, $P = 0.369$ at t_3, $P = 0.243$ at t_4, $P = 0.000$ at t_5, $P = 0.000$ at t_6).

Discussion

The findings of this study demonstrated the NAC pretreatment reduced the CBP-induced oxidoinflammatory response, possibly by preventing the oxidoinflammatory cascade activation, as indicated by the marked attenuation of MPO elevation, MDA formation, IL-6 release, and acute phase reactants. The activation of

neutrophils and the subsequent release of inflammatory mediators during cardiac surgery with the use of CPB are well recognized.[17,18] Reperfusion of the ischemic myocardium leads to the retention of leukocytes within vessels and their extravascular emigration.[19,20] Possible sites of leukocyte activation are complement activation via the classic pathway (heparin, surgery, and protamine-heparin complexes), the alternative pathway (blood-circuit contact), and the coagulation, kallikrein, and fibrinolytic cascades. The activation of leukocytes leads to the release of reactive oxygen or nitrogen species, granular enzymes, and arachidonic acid metabolites. Furthermore, endotoxins released from ischemic gut during CPB elicit the release of cytokines, such as tumor necrosis factor-α and IL-1, causing an inflammatory response.[19,21,22] All of these factors are thought to contribute to pump-induced damage, which was biochemically demonstrated by the enhanced production of the prototypical acute-phase proteins such as AAGP and CRP. Accordingly, we found that NAC pretreatment inhibited AAGP and CRP synthesis, and observed a relationship between these acute-phase proteins and IL-6 during CPB. Preventing the increase in IL-6 levels during CPB may help to suppress the AAGP and CRP levels, as IL-6 is well known for its capability to induce the production of acute-phase proteins by the liver.[23]

The high levels of MPO activity clearly demonstrated the activation of neutrophils during CBP, and the MDA levels increased after this activation. In our study, the peak level of MPO activation and MDA formation in the t_4 and t_5 time intervals demonstrated the role of the pump, and NAC pretreatment prevented these increased levels. We speculate that the possible mechanisms of lipid peroxidation inhibition by NAC were the prevention of neutrophil activation and infiltration, as indicated by the MPO activity, and the prevention of cytokine release, as indicated by the IL-6 levels. Our findings concur with those of previous studies documenting the inhibitory effect of NAC on neutrophil activation, adhesion molecule expression, lipid peroxidation, and cytokine production.[11–13]

Clinical trials have shown that NAC supplementation reduces oxidative stress by improving the thiol redox status.[24,25] Ortolani et al. recently reported an increase in reduced glutathione levels and prevention of lipid peroxidative damage when NAC was given to patients with septic shock.[25,26] In accordance with the previous findings, we found that the long-term administration of NAC inhibited lipid peroxidation in our study group. We also observed that NAC treatment attenuated MPO elevation, thus limiting the severity of the pump-induced inflammatory response. Moreover, Cuzzocrea et al. reported a decrease in MPO activity in the lung and ileum with NAC treatment, supporting our findings.[27,28] These results may highlight the importance of neutrophil activation and lipid peroxidation in the development of pump-induced oxidoinflammatory response.

The prevention of CPB-induced oxidoinflammatory response with long-term NAC pretreatment is relatively new. Thus, we believe that NAC pretreatment may be of great therapeutic value to help prevent pump-induced end-organ damage. Further clinical studies are needed to demonstrate the antioxidant and anti-inflammatory effects of NAC on the clinical parameters of patients with comorbidity and other risk factors, and those requiring complex heart surgery.

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