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Blood Cardioplegia with N-Acetylcysteine May Reduce Coronary Endothelial Activation and Myocardial Oxidative Stress

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ABSTRACT

Objectives: The aim of this prospective study was to compare the efficacy of intermittent antegrade blood cardioplegia with or without n-acetylcysteine (NAC) in reducing myocardial oxidative stress and coronary endothelial activation.

Methods: Twenty patients undergoing elective isolated coronary artery bypass graft surgery were randomly assigned to receive intermittent antegrade blood cardioplegia (32°C-34°C) with (NAC group) or without (control group) 300 mg of NAC. For these 2 groups we compared clinical outcome, hemodynamic evolution, systemic plasmatic levels of troponin I, and plasma concentrations of malondialdehyde (MDA) and soluble vascular adhesion molecule 1 (sVCAM-1) from coronary sinus blood samples.

Results: Patient demographic characteristics and operative and postoperative data findings in both groups were similar. There was no hospital mortality. Comparing the plasma levels of MDA 10 min after the aortic cross-clamping and of sVCAM-1 30 min after the aortic cross-clamping period with the levels obtained before the aortic clamping period, we observed increases of both markers, but the increase was significant only in the control group ($P = .039$ and $P = .064$ for MDA; $P = .004$ and $P = .064$ for sVCAM-1). In both groups there was a significant increase of the systemic serum levels of troponin I compared with the levels observed before cardiopulmonary bypass ($P < .001$), but the differences between the groups were not significant ($P = .570$).

Conclusions: Our investigation showed that NAC as an additive to blood cardioplegia in patients undergoing on-pump coronary artery bypass graft surgery may reduce oxidative stress and the resultant coronary endothelial activation.

INTRODUCTION

Ischemia-reperfusion injury may occur during several clinical situations, including as a consequence of suboptimal myocardial protection during heart surgery. Oxidative stress has a major role in the physiopathology of myocardial and endothelial dysfunction resulting from ischemia-reperfusion injuries [Laude 2001]. Therefore, studies have been proposed to investigate strategies to reduce oxidative stress for protection from injury not only of the myocardium but also of the coronary endothelium [Moukarbel 2004].

N-acetylcysteine (NAC), a relatively safe and extensively used drug with known antioxidant properties [Cotgreave 1997] was shown to reduce infarct size [Sochman 1996] and to protect the endothelium from oxidative stress [Cuzzocrea 2000, Rodrigues 2004]. Therefore, we believe that NAC has potential as an adjuvant in myocardial and endothelial protection during cardiac operations with cardiopulmonary bypass (CPB).

In this prospective study we compared the efficacy of intermittent antegrade blood cardioplegia with or without NAC in preserving myocardial and the endothelial function of patients undergoing elective isolated coronary artery bypass graft surgery (CABG). For this purpose we compared perioperative hemodynamic evolution, clinical outcome, coronary sinus plasma levels of malondialdehyde (MDA) (an indicator of lipid peroxidation), and soluble vascular adhesion molecule 1 (sVCAM-1) (an indicator of endothelial activation), as well as the systemic plasma levels of troponin I. Our rationale for using NAC is that its actions [Zafarullah 2003] may reduce the deleterious effects of the oxidative stress on the myocardium and coronary endothelium.

PATIENTS AND METHODS

Patients

After our study was approved by the ethics committee of our institution, 20 patients undergoing elective isolated CABG were randomly assigned to receive intermittent antegrade blood cardioplegic solution with (NAC group) or without (control group) NAC. In advance of starting the study we generated random treatment allocations in sequential numbers, each number corresponding to 1 of the 2 groups. Exclusion criteria were urgent or emergency procedures, combined procedures, reduced left ventricular ejection fraction of less than 35%, recent (within <4 weeks) myocardial infarction, and acute coronary syndromes.

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Cardiopulmonary Bypass, Myocardial Protection, and Operative Techniques

CPB was instituted by cannulation of the distal ascending aorta and insertion of a single 2-stage cannula into the right atrium. A membrane oxygenator (model Vital; Nipro, Sorocaba, Brasil) was used. Intravenous heparin (300 IU/kg) was administered immediately before cannulation for CPB, and additional doses were given to maintain an activated clotting time of 480 seconds or greater. Nonpulsatile flow rates of 2.2 to 2.4 L/min per m^2 and temperatures between 34°C and 37°C were used. The mean arterial pressure was maintained between 50 and 60 mmHg, with administration of sodium nitroprusside or norphenylephrine as required, and the haematocrit was kept higher than 25% by adding concentrated red blood cells if necessary. A 14F retrograde coronary sinus perfusion catheter (Edwards Lifesciences, Irvine, CA, USA) was inserted by palpation of the coronary sinus for blood collection for retrieving coronary sinus blood samples.

The basic cardioplegic solution used in both groups was prepared by mixing 500 mL of whole blood withdrawn directly from the pump oxygenator to a reservoir to which KCl (10 mEq), lidocaine hydrochloride (60 mg), and magnesium sulphate (8 mEq) were added. For the patients in the NAC group, 300 mg NAC (Fluimucil 300 mg/3 mL, Zambon Laboratories, São Paulo, Brazil) was added to the basic solution (3.676 mmol/L of NAC). Therefore, the cardioplegic solution for both groups had the same hematocrit and temperature of the systemic blood during CPB.

After cross-clamping of the ascending aorta, cardioplegia was induced in all patients by antegrade infusion of 500 mL of the cardioplegic solution (temperature of 34°C-37°C) obtained by use of a roller pump. Additional doses of 500 mL were infused after each distal anastomosis, or after 20 minutes of ischemia, and immediately before releasing the aortic cross-clamp.

All operations were performed through a median sternotomy, and all anastomoses were completed during a single period of aortic cross-clamp. At the end of the surgical procedure, protamine sulfate was administered to reverse the heparin effect.

Biochemical-Marker Assessment

Blood samples from the coronary sinus were collected to determine the serum levels of MDA before and 10 minutes after aortic clamping, and of sVCAM-1 before and 30 minutes after aortic clamping. The blood samples were centrifuged at 2700 rpm, and the plasma was stored at –70°C for further analysis. The sVCAM-1 concentration was determined by enzyme-linked immunosorbent assay (Human sVCAM-1 ELISA assay kit; R&D Systems, Minneapolis, MN, USA). The level of MDA was determined by spectrophotometry (Beckman DU640D spectrophotometer, 586 nm wavelength) with a lipid peroxidation assay kit (Calbiochem Biosciences, La Jolla, CA, USA).

The plasma levels of troponin I (heparin-plasma) before and 6, 12, and 24 hours after aortic cross-clamp were determined by immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA) from blood samples collected from the central radial artery catheter.

Hemodynamic Measurements

All patients received a Swan-Ganz catheter for continuous cardiac output measurement (CCOmbo; Edwards Lifescience), connected to a Vigilance I® monitor (Edwards Lifescience). In addition, central venous pressure, systemic (standard radial artery catheter), and pulmonary arterial blood pressure were continuously assessed. The cardiac, pulmonary, and systemic vascular resistance index were calculated before CPB and 30 minutes, 1 hour, 2 hour, 6 hour, 12 hours, and 24 hours after CPB.

Postoperative Management, Data Collection, and Definitions

Relevant baseline characteristics, intraoperative details, and postoperative clinical outcome data were collected prospectively for each patient randomized into the study. Perioperative myocardial infarction was defined as a new Q wave or the disappearance of the R wave persisting on 2 consecutive postoperative electrocardiographic tracings and/or peak creatinine kinase–MB serum activity higher than 80 IU/L. Any death occurring up to 30 days after hospital discharge was considerate as hospital mortality.

Statistical Analysis

We assessed data normality with the Shapiro-Wilk test. In each group, for comparing troponin I levels and the hemodynamic data, repeated-measures ANOVA (2-way ANOVA) or its nonparametric counterpart were planned. Unpaired *t*-test or Mann-Whitney test was used to compare non–repeated-

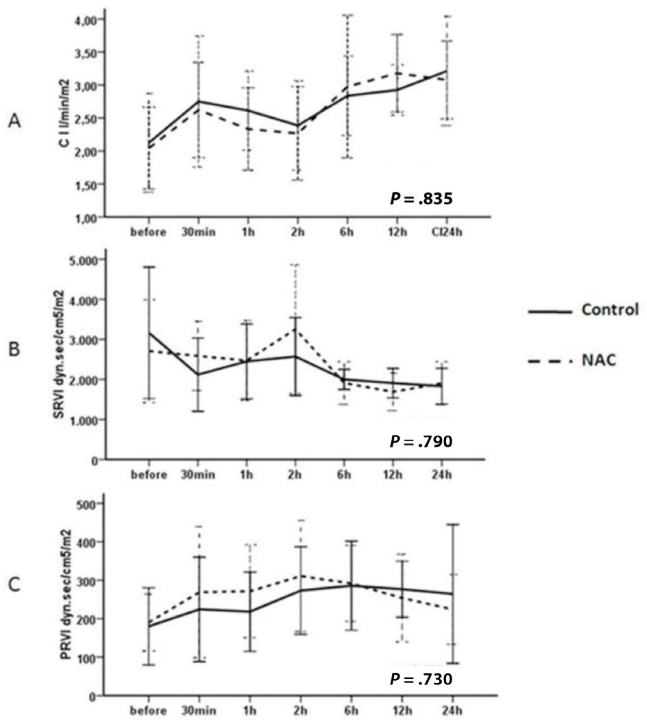

Figure 1. Hemodynamic parameters before and 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours, and 24 hours after cardiopulmonary bypass: cardiac index (panel A), systemic resistance vascular index (panel B), and pulmonary resistance vascular index (panel C). P values refer to between-group differences (2-way ANOVA for repeated measurements).

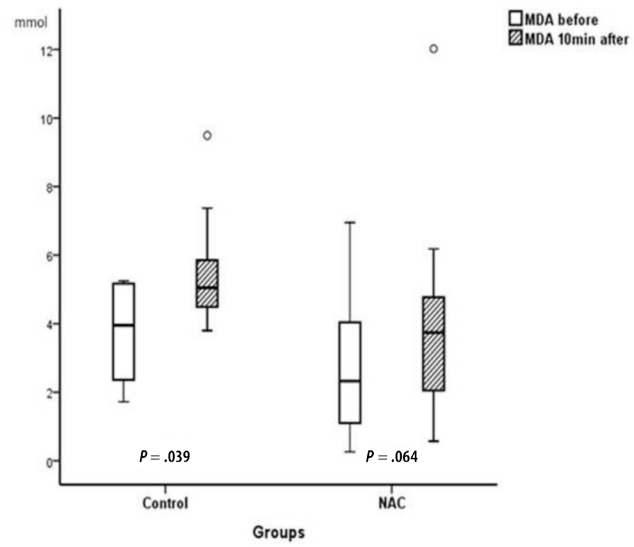

Figure 2. Plasmatic levels of malondialdehyde (MDA) from coronary sinus blood samples collected before and 10 minutes after releasing the aortic clamp. *P* values refer to comparison between before and after the aortic cross-clamp period in each group (Wilcoxon test).

measures data between the groups. Paired t-test or Wilcoxon test was used to compare sVCAM and MDA levels before and after aortic clamping within groups (in the same patient). For the comparison of categorical variables, the Fisher's exact test was used. Results are cited as mean ± SD, and a *P* value of less than .05 was accepted as significant.

RESULTS

Only the hemodynamic data and troponin I followed a normal distribution, according the Shapiro-Wilk test.

Demographics and Operative Data

Patient demographic characteristics and operative data findings in both groups were similar for all measured variables (Table). The differences were not significant. The volume of cardioplegic solution received by the control group was 2050 ± 166 mL and by the NAC group was 2050 ± 368 mL (*P* = .968). The dose of NAC infused in the NAC group was 1230 ± 221 mg (17 ± 4 mg/kg).

Postoperative Course

There was no hospital mortality. One patient in the NAC group had an ischemic stroke by postoperative day 5, and 1 patient in the control group had mediastinitis. The patients in both groups had similar hemodynamic evolution (Figure 1). Compared with before CPB, there was a significant decreasing of the systemic resistance vascular index (*P* < .001) followed by a significant increase of the cardiac index (*P* = .003) 6, 12, and 24 hours after CPB. The pulmonary vascular resistance index did not change significantly (*P* = .134).

Biochemical markers

Malondialdehyde. Compared with the levels observed before aortic cross-clamp, there was an increase in the serum level of MDA 10 minutes after release of the aortic clamp in both groups (Figure 2), but the increase was significant only in the control group (*P* = .034).

Soluble VCAM-1. Compared with the levels before aortic cross-clamp time, the sVCAM-1 levels 30 minutes after aortic clamp release increased in both groups (Figure 3), but the increase was significant only in the control group (*P* = .004).

Troponin I. In both groups we observed a significant increase of the systemic serum levels of troponin I 6, 12, and 24 hours after CPB (Figure 4) compared with levels observed before CPB (*P* < .001 for both groups) with higher concentrations occurring between 6 and 12 hours after CPB. The differences between both groups were not significant (*P* = .570).

COMMENTS

Our results revealed that after the aortic cross-clamp period there was an increase of the troponin I levels independently of the presence of NAC in the cardioplegic solution. On the other hand, the addition of NAC reduced the increase of sVCAM-1 and MDA observed in the coronary sinus effluent after the aortic cross-clamp period.

Since none of the patients presented with perioperative myocardial infarction, according the criteria previously defined, or had adverse hemodynamic evolution, the increase of the troponin I was probably due the inevitable operative cardiac tissue damage. However, because troponin levels may be considered an overly sensitive marker of cardiac injury in the setting of cardiac surgery, clinical interpretation of this increase may be difficult, mainly because of the different assay methods and isoforms used, resulting in a wide range of values reported [Croal 2006; Nesher 2008]. Nevertheless, in our study groups the lack of a significant difference in

Demographics and Operative Data*

	Control (n = 10)	NAC (n = 10)	*P*
Male sex, %	40%	60%	1.0
Age, y	53 ± 7	54 ± 11	.905
Ejection fraction, %	0.61 ± 0.1	0.59 ± 0.1	.965
LM obstruction	10%	10,0%	1.0
Diabetes	50%	40%	.656
COPD	10%	0%	.474
Peripheral vascular disease	0%	20%	.474
Systemic arterial hypertension	70%	100%	.211
Previous MI	33%	50%	.650
Aortic clamp time, min	69.7 ± 32	47.3 ± 16	.156
CPB time, min	91 ± 36	72 ± 21	.278
Distal anastomosis, n	3.1 ± 0.5	3.1 ± 0.3	.968

*LM obstruction, left main coronary artery obstruction; CPB, cardiopulmonary bypass; COPD, chronic obstructive pulmonary disease; MI, myocardial infarction.

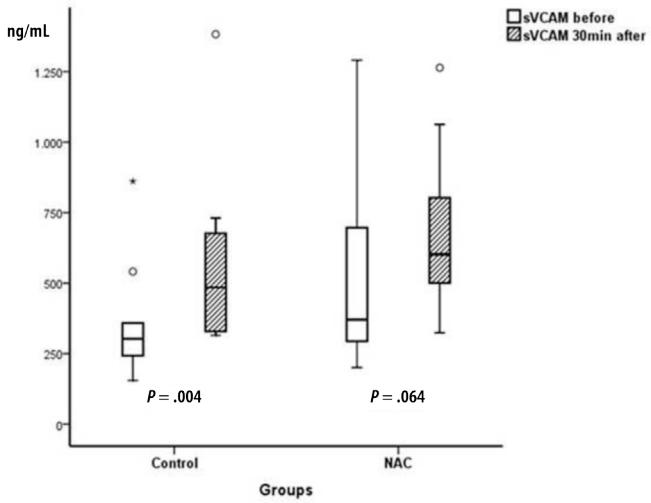

Figure 3. Plasmatic levels of soluble vascular adhesion molecule 1 (s-VCAM-1) from coronary sinus blood samples collected before and 30 minutes after aortic-clamp release. *P* values refer to comparison between before and after the aortic cross-clamp period in each group (Wilcoxon test).

troponin I levels, hemodynamic evolution, and outcomes indicates that both cardioplegic solutions had a similar effect in preserving the cardiac function.

However, even though both solutions equally preserved cardiac function in our investigation, they did differ in 2 aspects. The solution with NAC seemed to reduce oxidative stress and endothelial activation, judging by our data showing that the concentration of sVCAM-1 and MDA in the coronary sinus effluent before and after the cross-clamp time was not significantly different in the NAC group.

Other studies have investigated whether NAC may contribute to myocardial protection during cardiac surgery, each investigation using different concentrations of NAC, cardioplegic solution composition, and myocardial protection strategies [Vento 2003; Fischer 2003; Fischer 2004; Koramaz 2006]. Similarly to our results, these investigations, all based on similar rationales, showed several beneficial effects at the biochemical and cellular levels, but without apparent benefits in clinical outcomes.

Ischemia-reperfusion injury of the heart is not limited to cardiomyocytes but also extends to coronary vascular cells, resulting in decrease of the endothelium-dependent relaxation, especially that induced by NO. Such injury to the endothelium appears to depend on the increased production of oxygen-derived free radicals on reperfusion, leading to an increased degradation of NO and an acute inflammatory response characterized by increased adhesion of neutrophils to endothelial cells, and may be prevented by free radical scavengers [Papaharalambus 2007]. Therefore, the rationale for the protective effect of NAC during ischemia and reperfusion is mainly based on the concept that it acts as an antioxidant and as a glutathione substitute and precursor of glutathione, and regenerates endothelial function [Aruoma 1989; Zafarullah 2003].

Our results suggest that supplementing blood cardioplegic solutions with NAC may not only protect myocytes but may also provide better endothelial protection, resulting in less endothelial activation during on-pump CABG.

The levels of the sVCAM-1 and MDA we observed do not reflect exclusively endothelial activation of the coronary arteries and oxidative stress of the myocardium, but certainly the contribution of the myocardium and coronary endothelium in samples from the coronary sinus is preponderant. Therefore, even though the effects promoted by NAC on the levels of s-VCAM-1 and MDA from the coronary sinus effluent in the cardioplegic solution could be a result of systemic action, we believe this is improbable owing to the low systemic concentrations obtained with the doses we used compared to concentrations reported by other investigations [Vento 2003; Cakir 2004; Koramaz 2006].

We opted for a lower concentration of NAC and direct infusion in the coronary arteries, providing higher local concentrations, to reduce the chances of adverse events with higher systemic doses. In addition, high-dose NAC and glutathione repletion may up-regulate inducible nitric oxide synthase and may have a detrimental effect on epithelial cells exposed to hyperoxia [van Klaveren 1997; Groeneveld 2000].

In spite of the potential biochemical benefits of supplementing the blood cardioplegic solution with NAC, clinical benefits were not evident, because the clinical outcome and hemodynamic evolution were similar in both groups in our study and others [Vento 2003; Cakir 2004; Koramaz 2006]. One reason may be that in spite of the biochemical benefits NAC simply does not have such beneficial effects on clinical outcomes because the relative role of protein-adhesion molecules and markers of lipid peroxidation after cardioplegic arrest in the clinical landscape is not perfectly understood [Boldt 1998; Postadzhiyan 2008]. Another reason may be the small sample size, which may limit the power for demonstrating differences in clinical outcomes, mainly in patients with functional reserve that is adequate to overcome the consequences of CPB and ischemia-reperfusion injury. Perhaps

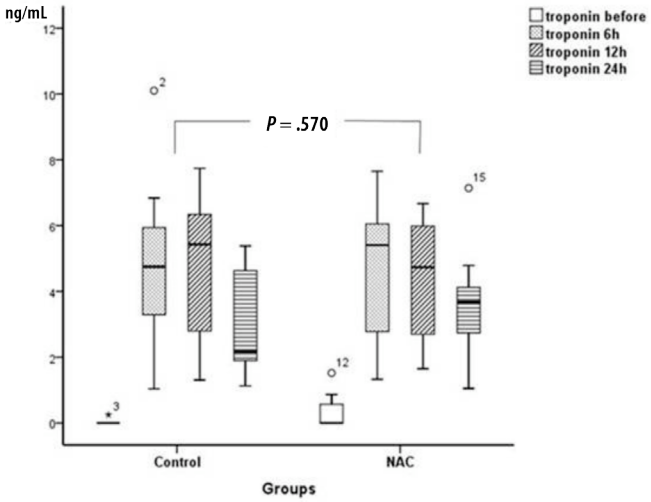

Figure 4. Plasma levels of troponin I from systemic blood samples collected before and 6, 12, and 24 hours after cardiopulmonary bypass. *P* values refer to between-group differences (2-way ANOVA for repeated measurements).

studies in larger samples, mainly in patients with limited functional reserve or in a more stressful clinical scenario, such as patients undergoing surgery during acute coronary syndromes, may reveal the clinical effectiveness of NAC. Demonstration of clinical benefits, however, may require large samples in clinical scenarios with low rates of adverse outcomes.

In conclusion, our investigation showed that NAC as an additive to blood cardioplegia in patients undergoing on-pump CABG surgery may reduce oxidative stress and resultant coronary endothelial activation.

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