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Antioxidant Therapy Reduces Oxidative and Inflammatory Tissue Damage in Patients Subjected to Cardiac Surgery with Extracorporeal Circulation

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Abstract: Ischaemia reperfusion injury is a pathophysiological event that occurs after cardiac surgery with extracorporeal circulation. This clinical event has been associated with the induction of oxidative and inflammatory damage in atrial tissue. Here, we tested whether combined omega 3 polyunsaturated fatty acids (n-3 PUFA)-antioxidant vitamin protocol therapy reduces oxidative and inflammatory cardiac tissue damage. This trial assigned 95 either-sex patients to supplementation with n-3 PUFA (2 g/day), or matching placebo groups, 7 days before on-pump surgery. Antioxidant vitamins C (1 g/day) and E (400 IU/day) or placebo were added from 2 days before surgery until discharge. Blood and atrial tissue samples were obtained during the intervention. Reduced/oxidized glutathione (GSH/GSSG) ratio, malondialdehyde (MDA) and protein carbonylation were determined in atrial tissue. Leucocyte count and high-sensitivity C-reactive protein (hs-CRP) in blood plus nuclear factor (NF)-κappaB activation in atrial tissue served for inflammation assessment. Lipid peroxidation and protein carbonylation were 27.5 and 24% lower in supplemented patients ($p < 0.01$). GSH/GSSG ratio was 38.1% higher in supplemented patients compared with placebo ($p < 0.01$). Leucocyte count and serum hs-CRP levels were markedly lower throughout the protocol in supplemented patients ($p < 0.01$). Atrial tissue NF-κB DNA activation in supplemented patients was 22.5% lower than that in placebo patients ($p < 0.05$). The combined n-3 PUFA-antioxidant vitamin protocol therapy here proposed reduced the oxidative stress and inflammation biomarkers, in patients undergoing on-pump cardiac surgery.

Cardiopulmonary bypass (CPB) with cardioplegic arrest unavoidably determines myocardial oxidative stress [1], inducing the release of reactive oxygen species (ROS) in the myocardium as part of the ischaemia reperfusion event. Oxidative stress also could generate structural and electrical modifications that would be a substrate for the cardiac dysfunction. In this regard, patients developing reperfusion arrhythmias have increased systemic and myocardial oxidative stress compared with those who remained in sinus rhythm [2], and this condition is also characterized by increased inflammatory cytokines that can drive to enhance the production of myocardial ROS. Indeed, white blood cells are massively activated during CPB, and they release cytokines, proteases, metabolites of arachidonic acid (AA) and ROS both in the bloodstream as well as in the tissues [3]. In particular, there is increasing evidence of an association between heart failure and a variety of inflammatory biomarkers, such as high-sensitivity C-reactive protein (hs-CRP), TNF-α and IL-6. Furthermore, it would seem that patients having higher hs-CRP values preoperatively have an increased risk of postoperative cardiovascular events after on-pump coronary surgery [4,5]. In this view, atrial biopsy specimens from cases with lone postoperative atrial fibrillation (AF), a reperfusion arrhythmia, have shown the presence of inflammatory infiltrates, probably secondary to pro-inflammatory chronic systemic state in cardiac patients [6]. Although the relative strength of the association of inflammation and oxidative stress markers with AF remains unclear, currently the role of inflammation and oxidative stress on electrical remodelling is under investigation. On this line, some interventions with antioxidants and anti-inflammatory agents represent the most reasonable pathway in the clinical treatment aimed to reduce its incidence of AF [7]. Recently, the administration of omega 3 polyunsaturated fatty acids (n-3 PUFA) has shown protective effects against cardiovascular lethal events, dependent in part of the regulation of pro-inflammatory signalling pathways.

The aim of this study was to test the hypothesis that the combined n-3 PUFA-antioxidant vitamin protocol reduces oxidative and inflammatory tissue damage in patients subjected to cardiac surgery with extracorporeal circulation.

Materials and methods

Study design and patient selection. The protocol consisted a randomized, double-blind, placebo-controlled trial conducted in patients undergoing cardiac surgery with extracorporeal circulation.

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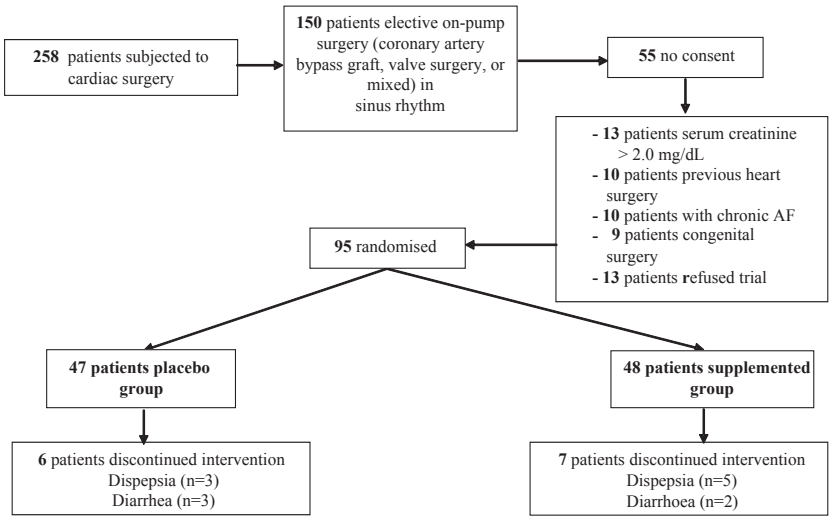

Fig. 1. Flow chart of screened and enrolled patients.

The eligible population was 150 either-sex patients, aged 30–80 years, scheduled for elective on-pump surgery (coronary artery bypass graft, valve surgery or mixed), all in sinus rhythm (fig. 1). The enrolment of patients took place at the Cardiovascular Department, University of Chile Clinical Hospital and Cardiothoracic Surgery Unit, San Juan de Dios Hospital, from August 2009 to January 2010. Exclusion criteria included documented chronic or paroxysmal AF or comorbidities such as congenital or previous heart surgery, advanced hepatic disease (cirrhosis) or chronic renal failure (serum creatinine >150 μM). Indeed, the patients with inflammatory or rheumatic diseases, corticosteroid users, those taking drugs having anti-inflammatory or antioxidant actions and patients that received previous 3-month fish oil supplements were excluded. Participants were analysed on the basis of demography and clinical characteristics (heart rate on resting prior surgery, risk factors, pharmacological treatment, ventricular ejection fraction, intraoperative and perioperative features) (table 1).This trial was conducted according to the Helsinki Declaration of the World Medical Association (2000). Ethical approval of the study was obtained from the Ethical Clinical Boards of each hospital. Informed written consent was obtained from all participants before any participation in the trial.

The participants were randomized, 7 days before surgery, to placebo or supplemented groups. Randomization was carried out centrally and was unstratified, block-based and computer-generated. Treatment was initiated immediately after randomization and consisted of daily doses of n-3 PUFAs (2 g/day) with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as ethyl esters in average 1/2 EPA/DHA ratio as previously described [8]. Two days before surgery, vitamin C (1 g/day) plus vitamin E (400 IU/day) were added. The whole supplementation regimen continued until hospital discharge. The placebo group received an equal number of capsules of identical size and colour, containing caprylic/capric triglyceride (825 mg per capsule), inert microgranules (500 mg) and vegetable oil (400 mg) replacing n-3 PUFAs, vitamin C and vitamin E, respectively [products provided by Procaps (Barranquilla, Colombia) and Gynopharm CRF (Santiago, Chile) Laboratories].

Continuous EKG monitoring after surgery was maintained in the cardiovascular intensive care unit 24–48 hr after finalizing surgery, to detect new onset AF. A Holter monitoring device was used until 4 days post-operation. Between the removal of Holter monitoring device and discharge, 12-lead EKG was utilized when arrhythmia symptoms occurred. Postoperative AF lasted at least 1 min. and confirmed by a monitoring system, EKG-Holter device or any symptomatic episode that required intervention, corroborated by 12-lead EKG.

Laboratory testing. Blood samples were drawn on enrolment (day −7), at the fifth day of n-3 PUFAs administration, before the addition of antioxidant vitamins (day −2), 15 min. before starting extracorporeal circulation (Time 0), 6–8 hr after finalizing the surgery (day +1) and on postoperative day 5 (day +5). Plasma supernatants and red blood cell lysates were stored at −70°C. Right atrial appendage samples (approximately 400 mg) were obtained immediately before starting extracorporeal circulation and frozen in liquid nitrogen and stored at −70°C. Biochemical determinations in atrial tissue from 29 patients (15 supplemented and 14 placebos) were not possible because of medical reasons or insufficient sample. Control atrial tissue samples were collected from patients subjected to cardiac surgery with extracorporeal circulation, without intervention (n = 8).

Oxidative stress-related parameters. The intracellular redox status in atrial tissue was assessed by a fluorometric method for measuring oxidized glutathione (GSSG) and reduced glutathione (GSH) [9]. The GSH/GSSG ratio was then calculated. Lipid peroxidation was measured through the determination of malondialdehyde (MDA), in plasma and atrial tissue samples by high performance liquid chromatography [10]. Protein carbonylation in atrial tissue was assayed by reacting 2,4-dinitrophenylhydrazine with protein carbonyls [11]. Results were expressed as nmol carbonyl/mg of protein.

Inflammation-related parameters. Inflammation was assessed through the measurements of plasma hs-CRP, by ELISA kit (Magiwell CRP kit, United Biotech; Mountain View, Santiago, Chile) [12] and leucocyte cell count.

Electromobility shift assay (EMSA). Nuclear protein extracts from atrial tissue samples were prepared according to Deryckere and Ganon [13]. The samples were subjected to EMSA for the assessment of NF-κB DNA binding using the NF-κB probe 5′-GATCTCAGAGGGGACTTTCCGAG-3′ (GrupoBios SA, Chile) labelled with α-^{32}P-dCTP using the Klenow DNA Polymerase Fragment I (Invitrogen Corp., Carlsbad, CA, USA), as previously described [14]. The specificity of the reaction was determined by a competition assay using 100-fold molar excess of unlabelled DNA probe. The subunit composition of DNA-binding protein was confirmed by supershift assay using specific antibodies from rat and rabbit IgG raised against NF-κB p50 and p65 (Santa Cruz Biotecnology, Santa Cruz, CA, USA). Samples were loaded on non-denaturing 6% polyacrylamide gels and run until the free probe reached the end of the gel; NF-κB bands were detected by autoradiography and quantified by densitometry using ImageJ (NIH Image, http://www.scioncorp.com, Scion Corporation, USA).

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Table 1.

Baseline characteristics of patients in protocol subjected to cardiac surgery with extracorporeal circulation.

	Placebo (n = 47)	Supplemented (n = 48)	*p*-value
Age, years	59 (10)	60 (13)	0.47
Sex (male/female)	36/11	32/16	0.50
BMI (kg/m^2)	24.7 (3.1)	25.6 (4)	0.11
Cardiac frequency (beats/min)	74 (11)	76 (11)	0.17
Left ventricular ejection fraction, %	54.2 ± 10.9	55.3 ± 11.7	0.65
Left atrial AP dimension, mm	45.6 ± 6.1	44.9 ± 5.2	0.76
Comorbidities			
Systemic hypertension, n (%)	15 (31.9)	18 (37.5)	0.92
Diabetes mellitus, n (%)	23 (48.9)	26 (54.1)	0.87
Hypercholesterolaemia, n (%)	22 (46.8)	23 (47.9)	0.98
Smoking history, n (%)	16 (34.0)	17 (35.4)	0.80
Perioperative features			
Revascularization, n (%)	23 (48.9)	25 (52.1)	0.51
Valve replacement, n (%)	16 (34.1)	14 (29.1)	0.90
Mixed aetiology, n (%)	8 (17.1)	9 (18.8)	0.89
Cross-clamp time, min.	77 (31)	78 (32)	0.28
CPB time, min.	91 (20)	89 (18)	0.77
Aspirin, n (%)	15 (31.9)	17 (35.4)	0.66
Atorvastatin, n (%)	18 (38.2)	17 (35.4)	0.89
Other statin, n (%)	6 (12.7)	7 (14.6)	0.30
ACE inhibitors, n (%)	26 (55.3)	29 (60.4)	0.21
ARBs, n (%)	9 (19.1)	11 (22.9)	0.93
Diuretics, n (%)	11 (23.4)	12 (25)	0.61
β-blockers, n (%)	29 (61.2)	28 (58.3)	0.82
Calcium channel blockers, n (%)	4 (8.5)	5 (10.4)	0.96
Nitrates, n (%)	2 (4.2)	2 (4.1)	0.58
Insulin, n (%)	3 (6.4)	1 (2.0)	0.54
Sulfanilylurea, n (%)	4 (8.5)	6 (12.5)	0.54
Biguanides, n (%)	10 (21.3)	12 (25)	0.91

AP, antero-posterior; CPB, cardiopulmonary bypass; ACE, Angiotensin I-converting enzyme; ARBs, Angiotensin II receptor blockers. Quantitative continuous variables are expressed as median (interquartile range) or mean ± S.D. if presented normal distribution, categorical variables are expressed and frequency (%).

Statistical analyses. The Shapiro–Wilk test and distribution plots were used to test the normality of distribution. For data that did not meet normality criteria, median and interquartile ranges were displayed. Two-sample Wilcoxon rank-sum test was used to evaluate the medians of variables. Descriptive statistics of continuous variables are presented as means ± standard deviation (S.D.) and compared by Student's *t*-test.

Relative risk (RR) was calculated for determining the probability of the AF occurring in the supplemented *versus* placebo group. Inflammation and oxidative stress-related parameters in plasma and atrial tissue were compared by repeated measures analysis of variance (RMANOVA). Indeed, the potential predictors of postoperative AF complications were evaluated in univariable analysis, and factors with $p < 0.05$ were then entered into a multivariable logistic regression analysis. $p < 0.05$ was considered statistically significant. Fisher's exact test was used to compare adverse event frequencies between supplemented and placebo groups. $p < 0.05$ was considered statistically significant. Statistical analysis was performed using Microsoft excel and Stata 10.00 for Windows.

Table 2.

Follow-up findings in treatment groups.

	Placebo (n = 47)	Supplemented (n = 48)	*p*-value
Postoperative AF, yes (%)	15 (31.9)	11 (22.9)	0.32
Duration AF, (min)	49 (10–100)	15 (1–240)	0.24
Hospitalization (days)	9.20 ± 0.18	8.15 ± 0.16	0.02
Postoperative complications, yes (%)	5 (10.6)	4 (8.3)	0.51

AF, atrial fibrillation.

Results

Study population.

The 95 study patients (68 men and 27 women) [mean age, 59 years (range, 41–81 years)] were included in the protocol. Demographic characteristics as well as pharmacological treatment of the patients included in the study are shown in table 1. No significant differences between the groups were found with respect to pharmacological treatment, comorbidities, left atrial antero-posterior dimension or duration of CBP.

Postoperative complications.

Postoperative AF occurred in 15 (31.9%) of 47 placebo patients *versus* 11 (22.9%) of 48 supplemented patients (RR = 0.32, CI 0.72–2.71, $p = 0.325$) (2.7 ± 0.2 *versus* 2.3 ± 0.1 days, respectively). Incidence of other postoperative complications was similar in both groups ($p = 0.73$, table 2). Four patients in the supplementation group (acute renal failure, n = 2; congestive heart failure, n = 1; and bleeding not requiring transfusion, n = 1) and five patients in the placebo group (acute renal failure, n = 2; mediastinitis, n = 2; and pneumothorax, n = 1) had postoperative complications. In the multivariable logistic regression analysis, non-predictors of AF were found.

Adverse events occurred in 13 randomized patients (supplemented *versus* placebo) (7 *versus* 6, $p = 0.52$). No further haemorrhagic or major adverse cardiovascular events (cardiovascular, non-fatal myocardial infarction or non-fatal stroke) were observed during 30 days of follow-up.

Oxidative stress-related parameters.

Oxidative damage of atrial tissue was lower in supplemented patients, as assessed by 27.5% lower MDA levels and 24% lower protein carbonylation, compared with supplemented patients ($p < 0.01$). The GSH/GSSG ratio in supplemented patients was 38.1 higher than that of who received placebo ($p < 0.01$) (table 3).

Inflammation-related parameters throughout the protocol are shown in fig. 2. Early after surgery (day +1), placebo group showed a significant increase in leucocyte count (fig. 2A) and serum hs-CRP (fig. 2B) reaching values 1.28 times ($p < 0.001$) and 17.5 times ($p < 0.001$) higher than its respective basal values. These changes were attenuated by supplementation. At discharge, supplemented patients showed leucocyte count and hs-CRP being 25.8 and 33.2% lower than placebo, respectively ($p < 0.05$).

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Table 3.
Inflammatory and oxidative stress-related parameters in patients subjected to antioxidant reinforcement.

	Placebo (n = 47)	Supplemented (n = 48)	*p*-value
GSH/GSSG ratio	7.63 ± 1.21	10.53 ± 0.66	<0.01
Atrial tissue MDA (μmol/mg protein)	4.32 ± 0.99	3.13 ± 0.83	<0.01
Atrial tissue carbonyls (nmol/mg protein)	3.22 ± 0.75	2.45 ± 0.75	<0.01
C-reactive protein (mg/dL)	2.02 (1.58)	1.56 (0.92)	0.18
Leucocyte count (cells/μL)	7200 (2040)	6500 (1830)	0.02

GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde.

Blood lipid peroxidation throughout the protocol is shown in fig. 3. After 5 days of n-3 PUFAs exposure, MDA levels were by 58% higher *versus* basal values ($p < 0.05$) and by 30% higher *versus* placebo values ($p < 0.05$). However, this difference was not apparent after further addition of antioxidant vitamins on Time 0. Early after finalizing surgery (day +1) and at the time of discharge (day +5), placebo exhibited 4 times and 1.7 times higher plasma lipid peroxidation levels than their respective basal and supplemented values, respectively ($p < 0.01$).

Atrial tissue DNA binding of NF-κB in protocol patients is shown in fig. 4. The higher intensity in EMSA bands in the atrial tissue of control patients is in agreement with previous data showing higher activation of this transcription factor under pro-inflammatory conditions [15]. Atrial tissue NF-κB DNA binding in placebo patients was comparable to values of DNA binding in the control patients, whereas supplemented patients showed DNA binding being 30.5 and 22.5% lower than that of control and placebo patients, respectively ($p < 0.05$) (fig. 4A). Suppression of the EMSA NF-κB in control (fig. 3C) bands by 100-molar excess of the respective unlabelled DNA probes confirmed the specificity of the determinations. Supplemented patient in supershift analysis shows both components of NF-κB rel p50 and p65 that are implicate in DNA binding and pro-inflammatory gene regulation (fig. 4D).

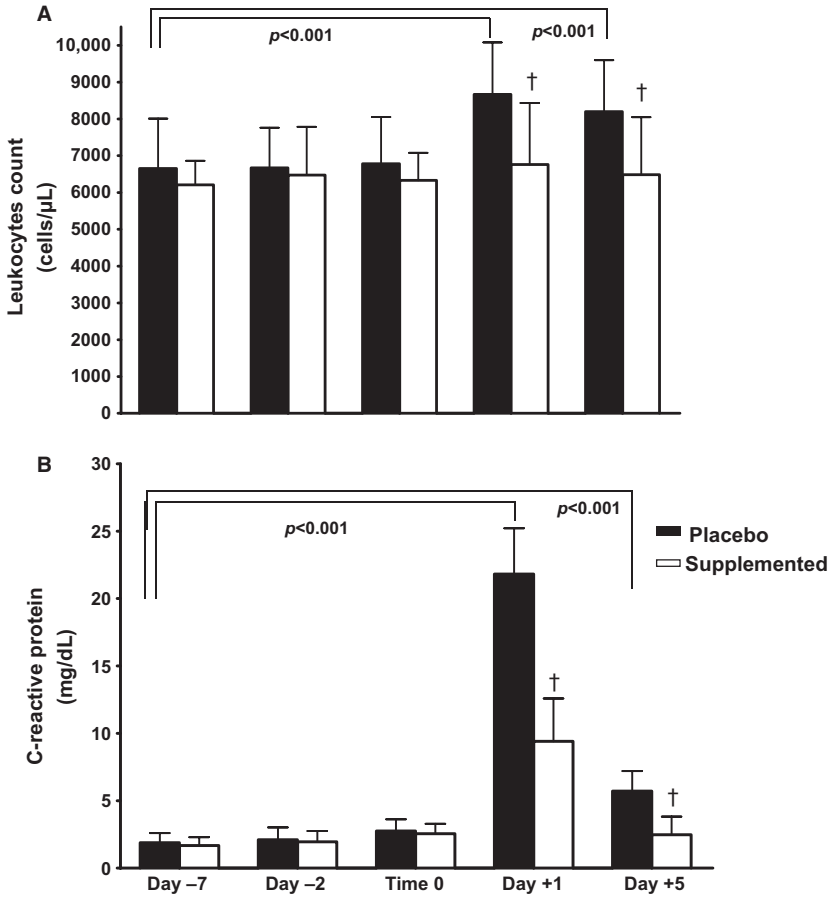

Fig. 2. Graphs representing inflammation-related parameters expressed by leucocyte count (A) and plasma high-sensitivity C-reactive protein (B) of placebo patients (n = 47) and patients undergoing supplementation with omega 3 polyunsaturated fatty acids plus antioxidant vitamins C and E (n = 48). Data presented show values at the moment of randomization (day −7), before the addition of antioxidant supplementation or placebo (day −2), on the day of surgery (Time 0), early after finalizing surgery (day +1) and on the day of discharge (day +5). Significant differences ($p < 0.05$): †*versus* placebo.

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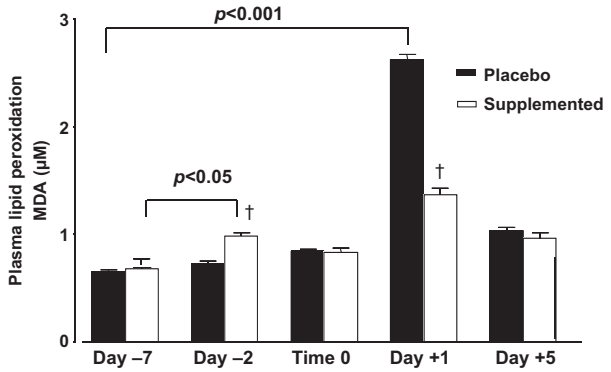

Fig. 3. Graphs representing the oxidative stress expressed by malondialdehyde levels in plasma of placebo patients (n = 47) and patients undergoing supplementation with omega 3 polyunsaturated fatty acids plus antioxidant vitamins C and E (n = 48). Data presented show values at the moment of randomization (day −7), before the addition of antioxidant supplementation or placebo (day −2), on the day of surgery (Time 0), early after finalizing surgery (day +1) and on the day of discharge (day +5). Significant differences ($p < 0.05$): †*versus* placebo.

Discussion

This study confirms that ischaemia reperfusion induced by cardiac surgery with CPB is associated with oxidative and inflammatory damage in atrial tissue. Therefore, it could be thus explained that patients subjected to combined n-3 PUFA-antioxidant vitamin protocol therapy evidence a lower inflammatory and oxidative stress biomarkers in blood and atrial tissue.

After cardiac surgery and particularly after extracorporeal circulation, ischaemic phenomena are mandatory. This generates high concentration of ROS playing an important role in a number of injuries affecting not only the heart but also other organs [16]. In these conditions, oxidation of PUFAs of membrane phospholipids by ROS can cause membrane disintegration, mitochondrial dysfunction, calcium overload and changes that lead to cardiomyocyte cell death [17]. Moreover, extracellular redox state can mediate the activation of inflammatory and pro-oxidant pathways such as NF-κB, activating transcription factor-1 and cAMP-response element-binding protein [18]. The activation of these pro-inflammatory pathways in the cardiomyocyte contributes significantly in the pathophysiology of structural changes such as fibrosis and cardiac hypertrophy [19].

During CPB, massively circulating neutrophils transmigrate into the myocardium at the time of reperfusion; here, they represent an important source of cytokines, inflammatory mediators and ROS [3,20]. Patients who have a higher postoperative leucocyte count are significantly more likely to develop cardiac dysfunction, whereas the preoperative count has no power to discriminate a subgroup of individuals at higher risk of postoperative arrhythmias [21]. These findings are in agreement with the present data, because no differences in leucocyte count baseline values were found comparing both intervention groups and higher postoperative levels in patients who received placebo with supplemented patients (fig. 2A).

High-sensitivity CRP is probably the most reliable and reproducible among the inflammatory biomarkers. In various studies of CPB-related acute phase reaction, it was

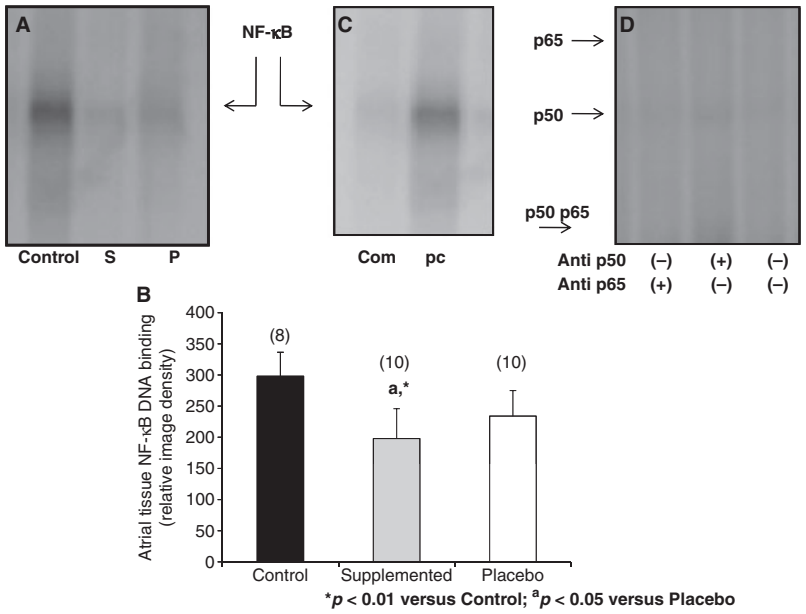

Fig. 4. Atrial tissue nuclear factor-κB (NF-κB) DNA binding on electromobility shift assay in control, supplemented and placebo patients. Autoradiograph representing lanes loaded with 8-μg nuclear protein in control (c), supplement (S), placebo (P) (A). Control patient in competition experiments without [positive control (pc)] and with 100-fold molar excess of the unlabelled DNA probe (com) (C), and supplemented patient in supershift analysis (D). Bar graphs corresponding to densitometric quantification of relative NF-κB DNA binding represent means ± S.D. for the number of patients indicated in parentheses (B). Significant differences ($p < 0.05$): **versus* control; [a]*versus* placebo.

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reported that the peak incidence of AF on the second to third postoperative day coincides with the peak of blood CRP [1,22]. In this study, the highest level of CRP was recorded at day +1, 6–8 hr after cardiac surgery (fig. 2B), and an association could be established between the surgical procedure, inflammatory response and the occurrence of cardiac dysfunction and postoperative AF. Recently, some studies have suggested that perioperative n-3 PUFA administration does not reduce the risk of AF after coronary artery bypass graft surgery, even at the same doses and identical placebo that were used in our study [23,24]. The complex interaction between circulating and tissue levels of these fatty acids, the effect of oxidative stress on free fatty acid release from adipocytes and the resulting changes in the electrophysiology of cardiomyocytes are not well understood and may explain much of the diversity in the outcome of clinical studies. Our data show that the occurrence of postoperative AF between both groups was similar (table 2).

The relative association of the occurrence of inflammation or oxidative stress with atrial remodelling is under investigation; it is known that a central response element of these two inciting causes is the NF-κB activation. NF-κB exists in its heterodimer state of the Rel protein family subunits p65 and p50. It remains inactive in the cell cytoplasm while bound to its repressor, inhibitory kBα (IkBα) [25]. ROS, cytokines and shear stresses resulting from reperfusion injury, all stimulate NF-κB via proximal kinase activation. Recent data show NF-κB activation in pro-inflammatory and pro-arrhythmogenic pathways, such as TGF-β, angiotensin II and transcriptional regulation of sodium and potassium channels in cardiac remodelling [26]. In the present study, the supplementation with n-3 and antioxidant vitamins diminished the activation of this factor (fig. 4), probably due to the attenuation of the inflammatory and pro-oxidant status occurring in protocol patients (fig. 2 and table 3).

Glutathione is the primary intracellular redox antioxidant. Some reports have shown that atrial glutathione content is lower in cardiac samples from patients subjected to cardiac surgery with CPB. This event is associated with oxidative stress occurrence and decreased cardiac contractile function [27]. The finding of these settings is in agreement with the glutathione depletion of the present study in the placebo patients (table 3). In contrast, supplemented patients may have glutathione repletion associated with up-regulation of signalling genomic pathways. The activation of antioxidant response element is triggered primarily by the binding of a transcription factor called nuclear factor (erythroid-derived 2)-like 2 (Nrf2). This mechanism is implicated in the antioxidant response characterized by increase in the transcription of phase II detoxification proteins and enzymes for glutathione biosynthesis, such as γ-glutamyl-cysteinyl ligase and glutathione reductase [28]. Furthermore, oxidized n-3 PUFA reacted directly with the negative regulator of Nrf2, Keap1, initiating Keap1 dissociation, thereby inducing Nrf2-directed gene expression [29].

Indeed, protective and anti-arrhythmic effects of supplementation could be attributed to anti-inflammatory actions of n-3 PUFAs. EPA and DHA can inhibit the production of inflammatory cytokines such TNF-α, IL-1β, IL-6 and IL-8 by monocytes, macrophages and endothelial cells, effects that are related to eicosanoid-dependent and eicosanoid-independent actions. For the eicosanoid-dependent actions, n-3 PUFAs are incorporated into inflammatory cell phospholipids at the expense of AA and hence inhibit AA metabolism to pro-inflammatory AA-derived eicosanoids. For the eicosanoid-independent actions, transcription factors modulate the balance between the pro- and anti-inflammatory cytokine productions. From this view, a new family of EPA and DHA-derived lipid mediators called resolvins and protectins has also recently been described [30]. They stimulate anti-inflammatory, pro-resolving and protective signalling pathways. Resolvin E1, for example, manifests its anti-inflammatory effects, in part, by decreasing NF-κB activation [31,32].

In turn, the beneficial effects of antioxidant vitamins C and E could also be owing to actions including those unrelated with their non-enzymatic antioxidant properties, such as down-regulation of NADPH oxidase activity, a major source of myocardial ROS during the ischaemia reperfusion cycle, and up-regulation of endothelial nitric oxide synthase [33].

It can be concluded that the antioxidant therapy reduces oxidative and inflammatory cardiac tissue damage. It is suggested that this design could be actively tested in further trials for its application as a suitable measure of improving the outcome of patients subjected to cardiac surgery.

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Conflict of interest

None of the authors or cooperative members has a proprietary, commercial of other financial interest in any study procedure or result.

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