L-arginine effects on myocardial stress in cardiac surgery: preliminary results

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Cardiac surgery; Coronary artery bypass graft; Ischemic heart disease; Myocardial protection.

Background. **L-arginine in addition to cardioplegia stimulates the release of nitric oxide and increases coronary blood flow, decreasing platelet activation and leukocyte adhesion. The aim of our study was to determine the feasibility and the efficacy of the addition of L-arginine to antegrade and retrograde blood cardioplegia in reducing myocardial damage and stress.**

Methods. **Twenty-eight consecutive patients who underwent coronary artery bypass grafting were randomized to receive 7.5 g of L-arginine in 500 ml of cardioplegic solution. To assess safety of use of L-arginine, hemodynamic evaluation was performed before sternum opening, at sternum closure, and 1 hour after arrival in the intensive care unit to measure cardiac index, systemic and pulmonary vascular resistances, and pulmonary capillary wedge pressure. Moreover, transesophageal echocardiography was performed to assess myocardial contractility. To determine the effects on myocardial stress, blood samples were taken from the retrograde coronary sinus catheter for lactate, interleukin (IL)-2 receptor, IL-6 and tumor necrosis factor (TNF)-α levels. Serum samples (preoperatively, 2, 18 and 42 hours after aortic cross-clamping removal) were also analyzed to measure creatine phosphokinase, creatine kinase-MB mass, cardiac troponin T, platelets, and leukocytes.**

Results. **We found statistical differences for IL-2 receptor, IL-6, TNF-α, platelets and leukocytes, in favor of the treated group, and decreasing trends in creatine kinase-MB mass and troponin T levels.**

Conclusions. **The present study shows the positive effects of the addition of L-arginine to cardioplegia. Reduced IL-2 receptor, IL-6 and TNF-α indicate a decrease in myocardial stress. Safety of L-arginine is related to lower values of systemic vascular resistances and pulmonary capillary wedge pressure observed in group A postoperatively that could improve the patient's outcome in terms of a reduced need for inotropic support. Moreover, the decrease in platelet and leukocyte count in the treated group might express a reduced no-reflow phenomenon and a better reperfusion, limiting endothelial injury from oxygen radical production.**

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Introduction

In order to achieve an improvement in myocardial protection, experimental trials studied the efficacy of L-arginine in addition to cardioplegic solution during cardiac arrest in cardiac surgery. Mechanisms of action should be related to the release of nitric oxide (NO) with increased coronary blood flow and to the decreased platelet activation and leukocyte adhesion properties[1,2].

Our clinical study was designed to test the efficacy of L-arginine added to cardioplegia in reducing myocardial damage as assessed by troponin T, creatine kinase (CK)-MB, creatine phosphokinase (CPK) and cytokine release. Hemodynamic and echocardiographic measurements were performed to verify safety of the use of this amino acid in cardiac surgery operations.

Methods

Between June 1 and August 31, 2003, all consecutive patients with coronary artery disease who underwent coronary artery bypass graft operation were randomized into two groups: group A treated with L-arginine in cardioplegic solution and group B as control. Informed consent was obtained and the study was approved by the institutional ethics committee. Patients > 80 and < 45 years were excluded. Patients with recent acute myocardial infarction (AMI) (< 10 days), patients who needed other surgical procedures, redo patients or patients who underwent emergency coronary artery bypass graft or had perioperative AMI, were excluded.

Myocardial protection. We used a cold intermittent antegrade and retrograde cardio-

plegia with warm induction[3-5] and moderate hypothermia (34°C). Blood 4:1 cardioplegic solution B1 (Table I) was administered at induction and for intermittent doses and blood cardioplegic 4:1 B2 (Table I) solution was used for reperfusion time.

In both groups cardiac arrest was achieved by delivering antegrade and retrograde blood cardioplegia with a first phase of warm induction (37°C) followed by a cold phase (4°C). The warm phase consisted in a period of 2.5 min of antegrade perfusion at 300-350 ml/min of high-potassium cardioplegic solution, followed by a 200-250 ml/min retrograde delivery for 1.5 min. The correct position of the retrograde cannula was verified indirectly measuring infusion pressure that was maintained between 25 and 40 mmHg. The second phase of induction deals with the cold infusion of a low-potassium blood cardioplegic solution. This solution is delivered by intermittent antegrade and retrograde infusions after performing each distal anastomosis. After the last distal anastomosis (usually the left internal thoracic artery on the left anterior descending artery), the reperfusion phase begins with a first antegrade perfusion 2 min long with the 4:1 blood B2 solution. Then a 1-min retrograde perfusion of B2 blood cardioplegia at a rate of 150-200 ml/min is delivered, followed by a whole blood washout phase.

Cardioplegia solution was prepared by one technician and all operatory room staff and analysts were blinded for the study.

All patients were randomized according to a random table so that group A received a dose of 7.5 g of L-arginine in 500 ml of cardioplegic solution in B1 and B2 solutions except for reperfusion time.

Anesthesia protocol. A total intravenous balanced anesthesia was used. Right heart catheterization through the internal jugular vein was used in all patients for hemodynamic assessment by a Swan-Ganz flotation catheter (Edwards 131 HF7).

ECG leads (D2 and V_5) were continuously recorded during surgical procedure and in the intensive care unit.

Surgical technique. All patients were operated on by the same experienced cardiac surgeon with the same technique of myocardial revascularization. The left internal thoracic artery was used to perform the anastomosis on the left anterior descending artery in all patients. The saphenous vein was used to perform other anastomoses. Complete arterial revascularization, using both the mammary arteries and the radial artery or the right gastroepiploic artery, was performed in patients < 70 years, non-insulin-dependent diabetes, not affected by obesity or chronic obstructive pulmonary disease.

Table I. Cardioplegic solution.

Component	B1 solution	B2 solution
Na^+ (mEq/l)	57.5	120.7
K^+ (mEq/l)	48.8	56.6
Cl^- (mEq/l)	83.2	56.6
P^- (mEq/l)	1.3	6.8
Citrate (mmol/l)	25.6	134
Anticoagulant CPD (ml)	225	225
Glucose 5% (ml)	250	250
Glucose 50% (ml)	0	40
KCl_2 (mEq/ml)	15	15

CPD = citrate phosphate dextrose.

Preoperative data. The following data were collected: age, extension of coronary disease, left main stenosis ≥ 50%, hypertension, previous stroke, insulin-dependent diabetes mellitus, previous AMI, recent AMI (< 30 days), chronic obstructive pulmonary disease, unstable angina, peripheral artery disease. Among the operative findings, we considered in the two groups extracorporeal circulation time and aortic cross-clamping time, the total amount of cardioplegic solution administered and the relative number of doses, the minimum temperature recorded, the mean number of grafts performed, the use of double internal thoracic arteries.

We studied the outcome of patients in terms of ECG lead modifications, postoperative inotropic drug use, mediastinal blood loss in the first 24 hours, atrial fibrillation, ventricular arrhythmias, and other postoperative complications, mechanical ventilation time, intensive care unit stay and hospital stay.

We did not consider as inotropic treatment the use of dopamine < 4 γ/kg/min.

Echographic, hemodynamic and chemical analysis. To assess safety of the use of L-arginine, all patients underwent a transesophageal echocardiographic study, before aortic cannulation and before protamine administration evaluating myocardial contractility, as expressed by ejection fraction. Also hemodynamic data were collected before sternum opening (time 0), at sternum closure (time 1), and 1 hour after the arrival in the intensive care unit (time 2), using the Swan-Ganz catheter to measure cardiac index, systemic vascular resistance index (SVRI), pulmonary vascular resistance index, and pulmonary capillary wedge pressure (PCWP).

To determine effects on myocardial stress, blood samples were collected from the retrograde coronary sinus catheter for lactate, tumor necrosis factor (TNF)-α, interleukin (IL)-2 receptor, IL-6 level measures before aortic cross-clamping (time 0), at aortic cross-clamping removal (time 1) and 15 min after (time 2). To assess myocardial damage serum samples from systemic blood vessels were also analyzed at four different times (preoperatively, 2, 18 and 42 hours after aortic clamping removal), in order to measure CPK, CK-MB mass, and cardiac troponin T. Platelet and leukocyte levels were also monitored, to verify effects on endothelial reperfusion damage.

Statistical analysis. All data were expressed as mean ± SD. Analysis of continuous variables was performed with the Student's t-test and multivariate analysis of variance test. Analysis of categorical variables was performed with the Fisher's exact test. ANOVA for repeated measures was employed to analyze modification between groups for different time points. A p value of ≤ 0.05 was considered as statistically significant.

Results

Twenty-eight patients were studied (15 in group A and 13 in group B). The two groups did not show any statistical differences in demographic and preoperative clinical findings (Table II) and can be considered homogeneous for operative data (Table III). Postoperative data showed statistical differences between the two groups in SVRI, PCWP, leukocyte and platelet count, TNF-α, IL-2 receptor, and IL-6 levels. All other findings were not significantly different in the two groups.

Table II. Preoperative data in the two study groups.

	Group A (n=15)	Group B (n=13)	p
Age (years)	63.93 ± 8.75	64.46 ± 9.4	0.88
No. diseased vessels	3 ± 0.5	3 ± 0.4	0.57
Hypertension	8 (53%)	6 (46.1%)	1
Stroke	2 (13.3%)	0	0.48
Peripheral arteriopathy	2 (13.3%)	1 (7.7%)	1
Left main stenosis	1 (6.6%)	3 (23%)	0.37
Diabetes	3 (20%)	2 (15.3%)	1
Previous AMI	7 (46.6%)	6 (46.1%)	1
Recent AMI	4 (26.6%)	2 (15.3%)	0.65
COPD	6 (39.9%)	2 (15.3%)	0.22
Unstable angina	8 (53%)	6 (46.1%)	1

AMI = acute myocardial infarction; COPD = chronic obstructive pulmonary disease.

Table III. Operative findings in the two study groups.

	Group A (n=15)	Group B (n=13)	p
ECC (min)	98 ± 20	106 ± 18	0.31
AC (min)	67 ± 17	80 ± 20	0.09
Cardioplegic solution (ml)	740 ± 134	769 ± 106	0.54
Doses of cardioplegia	3.1 ± 0.9	3.8 ± 0.9	0.06
Systemic minimum temperature (°C)	34.6 ± 0.4	34.7 ± 0.4	0.91
Grafts	2.6 ± 0.5	2.8 ± 0.4	0.16
Double ITA	6 (40%)	7 (53.8%)	0.70
Sequential grafts	1 (6.6%)	0	1

AC = aortic cross-clamping; ECC = extracorporeal circulation; ITA = internal thoracic artery.

A slight increase in SVRI was observed in group A (p = 0.046; Fig. 1A) whereas PCWP was remarkably decreased in this group (p = 0.006; Fig. 1B). Platelet count was significantly increased in the treated group (p = 0.015; Fig. 1C). Moreover, a postoperative decrease in leukocyte count was observed in this group (p = 0.044; Fig. 1D).

Blood samples from the coronary sinus cannula for lactate evaluation, showed no difference in the two groups.

Decreased values of TNF-α, IL-2 receptor and IL-6 were observed in group A after aortic cross-clamping removal (p = 0.002, p = 0.0027, and p = 0.014, respectively; Fig. 2).

There were not any statistical differences between the two groups in terms of release of CPK, CK-MB mass and troponin T although, as shown in figure 3, lower levels of these markers were found in group A.

Cardiac index did not show any significant difference between the two groups (Fig. 4).

Postoperative data did not show any significant difference between the two groups (Table IV), although there was a trend through an increase in the need for inotropic support and an increase in blood loss in the control group.

In all patients but one, myocardial activity after aortic cross-clamping removal restarted with sinus rhythm. The only patient who had an episode of ventricular fibrillation after aortic cross-clamping removal restored with CD-shock was in group A. Moreover, one episode of postoperative bronchoconstriction and one case of pleural effusion were observed in this group. In group B, 1 patient presented prolonged bronchoconstriction, and 1 patient had low output syndrome.

Discussion

The benefits of L-arginine administration on endothelial dysfunction have been considered in many experimental studies[5-7]. NO, a highly soluble gas produced by endogenous metabolism, is a mediator able to reduce the ischemia-reperfusion damage in animal experimental models[6]. It diffuses readily through cell phospholipid layers and it can diffuse also across the smooth muscle cell membrane and in the intravascular space. L-arginine is a NO donor and it has many mechanisms of action that protect against free radical oxygen injury[7]. Experimental studies suggested that the addition of L-arginine to blood cardioplegia could restore endothelial function by increasing NO production and integrating the homeostatic response[7].

The present study was designed to verify safety and efficacy of L-arginine added to cardioplegia. Safety was assessed with echocardiographic study that did not show any differences between groups demonstrating that this amino acid could be used without detrimental effects on myocardial contractility.

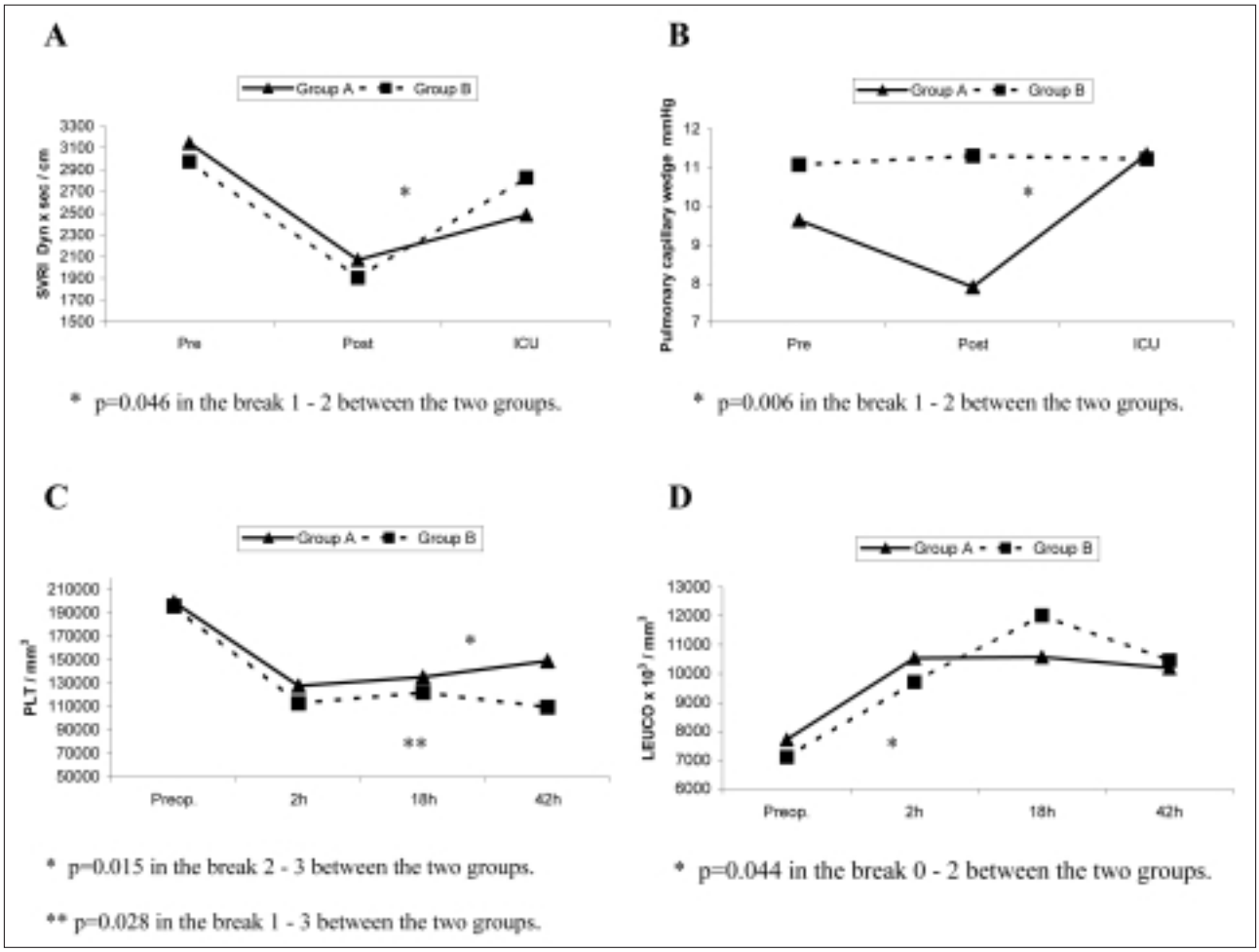

Figure 1. A: *systemic vascular resistance index (SVRI), times 0, 1, 2.* B: *pulmonary capillary wedge pressure, times 0, 1, 2.* C: *platelet (PLT) levels in the two study groups, times 0, 1, 2, 3.* D: *leukocyte (LEUCO) levels in the two study groups, times 0, 1, 2, 3. ICU = intensive care unit.*

Hemodynamic assessment showed statistical differences in SVRI and CPWP. The decrease of SVRI in group A after aortic cross-clamping removal might be due to a systemic vasodilatory effect of L-arginine as a precursor in NO production. Although the local administration of cardioplegia was limited to the coronary districts through the antegrade and retrograde catheters, part of this cardioplegic solution drained into the right atrium returns to the oxygenator to be infused again in the systemic circulation by the pump. The studies of Bode-Boger et al.[8] showed that systemic administration of L-arginine may decrease arterial constriction and consequently the left ventricular afterload. The decrease in SVRI reduces myocardial stress and may have benefits after the end of extracorporeal circulation. A study carried out by Ogilvie and Zborowska-Sluis[9] showed the link between L-arginine infusion and decrease in SVRI with an increase in cardiac output[9]. The trend of group A toward statistically decreased PCWP values could be a direct consequence of the lower SVRI. The improved hemodynamics due to L-arginine action is underlined in the present preliminary study and the trend toward a decrease in postoperative inotropic support might be consistent with these data.

We found a significant difference in postoperative platelet count (Fig. 3). Group A showed higher platelet levels than group B. It is a well known phenomenon that reperfusion process causes endothelial dysfunction and damage that may result in vasospasm, platelet activation, leukocyte adhesion and capillary obstruction, increased oxygen radical production, with prothrombotic effects. NO improves endothelial function, decreases platelet adherence leading to an increase in capillary blood flow[10]. Lower platelet consumption in our patients treated with L-arginine may be the consequence of these protective mechanisms. A clinical effect of this reduction in platelet consumption could be the decreased blood loss reported in the treated group.

In our study we found a difference in leukocyte count between the two groups, showing a more important postoperative increase in the control group. NO biochemical actions during the critical period of reperfusion, decrease oxygen radical injury (mainly produced by neutrophils) because of a decreased neu-

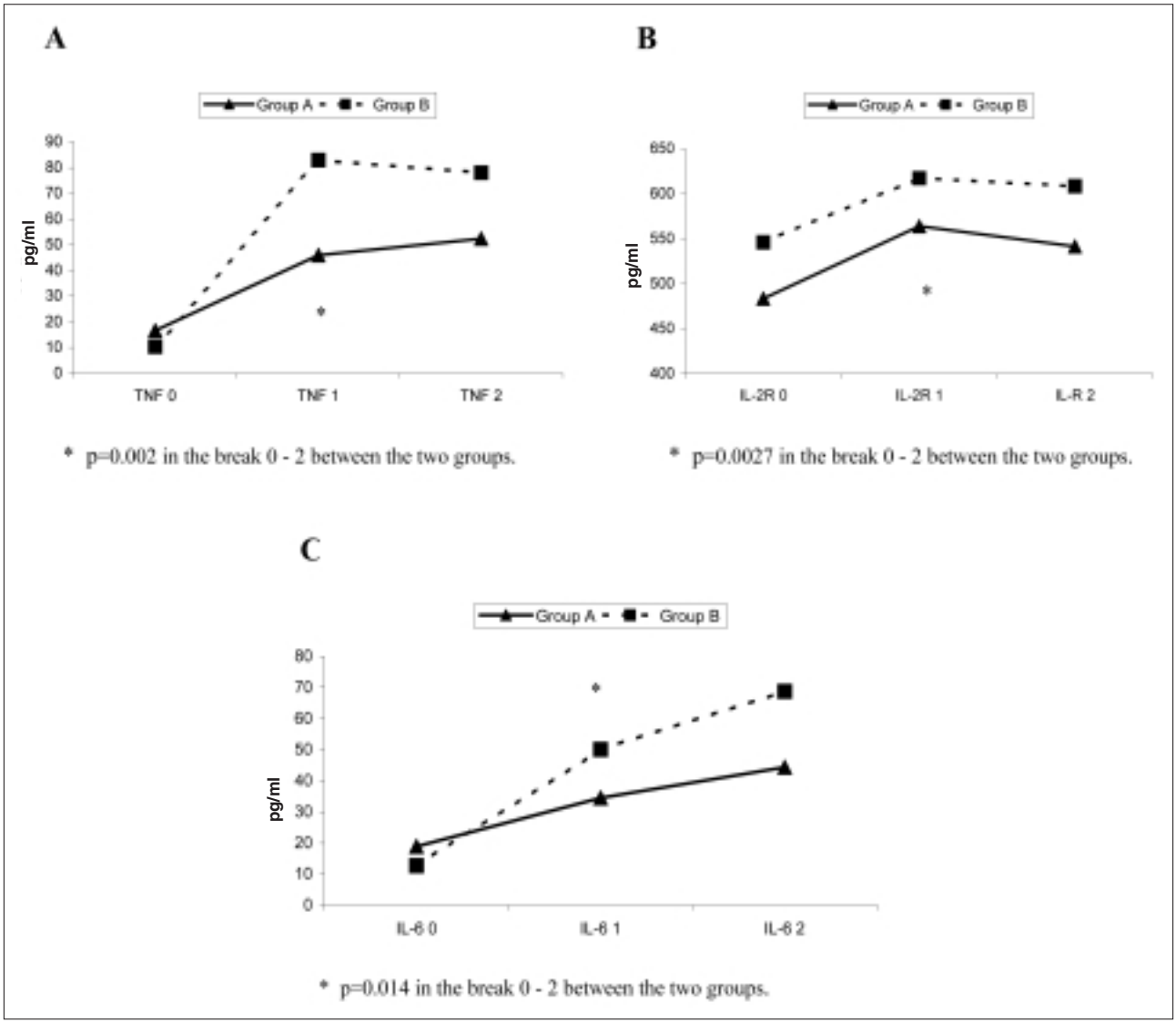

Figure 2. A: *tumor necrosis factor (TNF)-α levels before, at aortic cross-clamping removal, and 15 min later. The increase was lower in group A.* B: *interleukin-2 receptor (IL-2R) levels before, at aortic cross-clamping removal, and 15 min later.* C: *interleukin-6 (IL-6) levels before, at aortic cross-clamping removal, and 15 min later.*

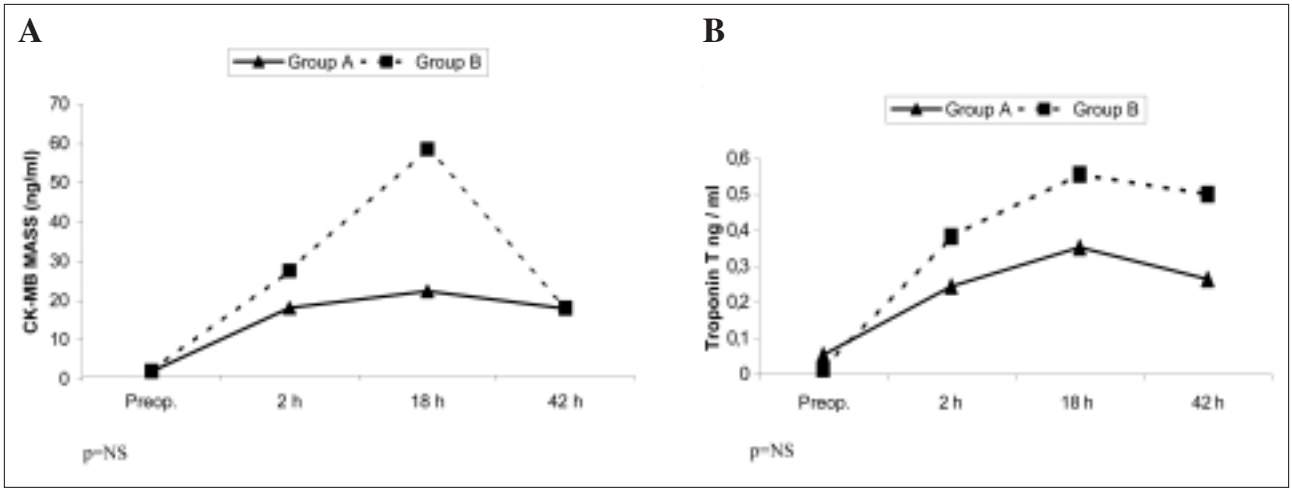

Figure 3. *Creatine kinase (CK)-MB mass* (A) *and troponin T* (B) *levels, times 0, 1, 2, 3 (preoperatively, 2, 18, and 42 hours after aortic cross-clamping removal).*

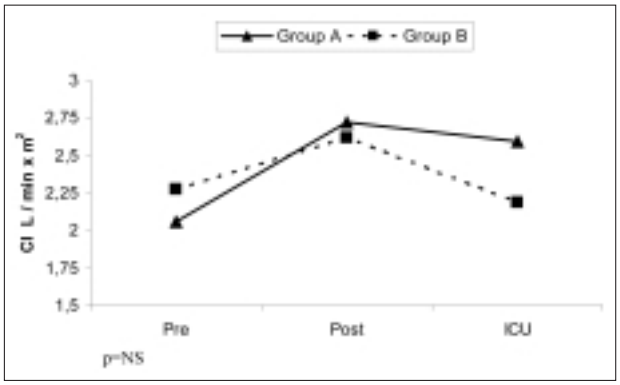

Figure 4. *Cardiac index (CI). ICU = 1 hour after arrival to the intensive care unit; Post = before protamine administration; Pre = before aortic cannulation.*

Table IV. Postoperative data in the two study groups.

	Group A (n=15)	Group B (n=13)	p
Use of inotropes	5 (33%)	9 (69%)	0.13
Bleeding (ml)	734 ± 334	976 ± 284	0.55
Intubation time (hours)	13.4 ± 8.3	22.0 ± 21.7	0.18
ICU stay (hours)	26.0 ± 8.9	41.1 ± 28.2	0.07
Hospital stay (days)	5.6 ± 1.2	5.8 ± 1.8	0.73
Atrial fibrillation	6 (40%)	4 (31%)	0.69
Complications	2 (14%)	2 (15%)	1

ICU = intensive care unit.

trophil and platelet adherence[11]. A direct measure of myeloperoxidase activity and oxygen radical production may give more information.

The aim of this study was to determine the efficacy of L-arginine in reducing myocardial stress and damage in terms of myocardial cytokines, troponin T and CK-MB release. There were statistical significant differences in TNF-α, IL-2 receptor, and IL-6 measures supporting a better myocardial preservation with L-arginine. Recent experimental studies suggested that the cytokines expressed in the myocardium in response to environmental injury, play an important role in the homeostatic responses. They have the potential to produce myocardial damage in high concentration, though their short-term expression may be part of an adaptive response to stress[12]. Many studies suggested that the myocardium is a TNF-α producing tissue especially in response to ischemia-reperfusion injury[13,14]. In this study we observed a decrease in TNF-α, IL-2 receptor, and IL-6 levels between the groups. These results are encouraging and demonstrate that L-arginine could be a protective agent against myocardial injury in cardiac surgery.

Carrier et al.[15] using L-arginine in blood cardioplegia in the first clinical trial reported a significant decrease in troponin T and CK-MB mass levels. In our study we observed a decreasing trend for CK-MB mass and troponin T in group A, though not statistically significant. The limited number of patients considered in this preliminary study and our myocardial protection strategy that has already optimal results, may however limit the possibility of reaching better levels in terms of reduction of these myocardial damage markers.

Study limitation. The limitation of our study is related to the small number of patients and a larger population needs to be further investigated to confirm our preliminary results.

In conclusion, in this study, we could identify some positive effects of the addition of L-arginine to cardioplegic solution. The use of L-arginine leads to a reduction in myocardial TNF-α, IL-2 receptor, and IL-6 levels, demonstrating a decrease in myocardial stress during cardiac arrest. Larger trials are needed to assess real benefits in terms of reduction of minor myocardial damage, as expressed by troponin T and CK-MB mass release.

We demonstrated the feasibility and safety of L-arginine in addition to cardioplegia in humans. The vasodilatory effects, as those observed in group A, could improve the patient's outcome. Moreover, in our study we found systemic benefits (for SVRI, platelet and leukocyte count) due to L-arginine actions. On the basis of these findings, it might be useful to consider a systemic administration of L-arginine to test its efficacy against extracorporeal circulation injuries.

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