Effects of L-Arginine Administration Before Cardioplegic Arrest on Ischemia-Reperfusion Injury

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Background. **Administration of L-arginine during reperfusion or its addition to cardioplegic solution has been shown to protect myocardium against ischemia-reperfusion injury. This study aimed at evaluating the role of L-arginine in ischemia-reperfusion injury when administered intraperitoneally 24 hours before cardioplegic arrest.**

Methods. **Two groups of Sprague-Dawley rats (control, n = 10; and L-arginine, n = 10) were studied in an isolated buffer-perfused heart model. Both groups were injected intraperitoneally 24 hours before ischemia. Before experimentation blood samples were collected for cardiac troponin I and cGMP analysis. In the coronary effluents, cardiac troponin I, adenosine, cyclic guanosine monophosphate, and nitric oxide metabolites were assayed.**

Results. **Before heart excision, serum cardiac troponin I concentrations were higher in the L-arginine than in the control group (0.037 ± 0.01 versus 0.02 ± 0.05 $\mu g \cdot L^{-1}$; $p < 0.05$). During reperfusion, cardiac troponin I release was lower in the L-arginine than in the control group (0.04 ± 0.01 versus 0.19 ± 0.03 $ng \cdot min^{-1}$; $p < 0.05$). The coronary flow as well as the left ventricular developed pressure were higher in the L-arginine than in the control group before ischemia and remained so throughout the experimentation.**

Conclusions. **These results indicate that L-arginine administered intraperitoneally 24 hours before cardioplegic arrest reduced myocardial cell injury and seems to protect myocardium against ischemia-reperfusion injury.**

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Nitric oxide (NO) is a vasodilatory molecule synthetized from L-arginine by NO synthase (NOS) and released continuously by endothelial cells [1]. Ischemia-reperfusion has been shown to injure cardiomyocytes and coronary endothelium [2]. Endothelial dysfunction induces a decrease in NO release during reperfusion and therefore affects its modulator properties such as vasodilatory, platelet adherence and neutrophil function [2]. Administration of L-arginine during reperfusion or its addition to cardioplegic solution has been shown to protect myocardium against ischemia-reperfusion injury and to improve the coronary flow by increasing NO levels [3–5]. The effects of L-arginine on ischemia-reperfusion injury, when given before ischemia and intraperitoneally (IP), are unknown.

The aim of this study was to test if L-arginine administered IP 24 hours before ischemia could induce pharmacologic cardioprotection against ischemia-reperfusion injury with the three more specific objectives to determine (1) the release kinetics of NO metabolites, cyclic guanosine monophosphate (cGMP), adenosine, and cardiac troponin I (cTnI) during ischemia and reperfusion; (2) the effects of L-arginine on postischemic myocardial variables; and (3) whether these biochemical events participated in the observed effect during reperfusion. Intraperitoneal administration of L-arginine was chosen because this route of delivery has been shown to induce citrulline production and therefore NO formation in rat heart by the increase in L-arginine concentration in the heart [6]. To the best of our knowledge, there are no similar data for intravenous administration.

Material and Methods

Isolated Rat Heart Perfusion

Male Sprague-Dawley rats (Charles River, Saint Aubin Les Elbeuf, France) weighing 270 to 320 g were anesthetized with an IP injection of sodium pentobarbital (60 $mg \cdot kg^{-1}$) and heparinized intravenously with heparin sodium (1,000 $UI \cdot kg^{-1}$). All animals received humane care in compliance with the "Guide for the Care and Use of Laboatory Animals" (National Institutes of Health publication No. 85-23, revised 1985). Hearts were rapidly excised and placed in ice-cold perfusion buffer. After aortic cannulation the hearts were perfused retrogradely in the Langendorff mode at a constant pressure of 80 mm Hg and were permitted to beat at intrinsic heart rate throughout experimentation.

The perfusion solution was a modified Krebs-Henseleit buffer (K-H) containing (in $mmol \cdot L^{-1}$): NaC1 118; $NaHCO_3$ 25; KC1 3.2; $MgSO_4$ 1.6; KH_2PO_4 1.2; $CaCl_2$ 2.5, and glucose 5.5. All perfusion solutions were passed through a 0.8 μm porosity filter (Millipore, Bedford, MA) to remove contaminants, gassed with 95% O_2, 5% CO_2, and adjusted at pH 7.4. The hearts were not paced and

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temperature was kept constant at 37°C during stabilization and reperfusion and at 20°C during ischemia. A water-filled latex balloon (No. 4, Hugo Sachs Electroniks, March-Hugstetten, Germany) connected to a pressure transducer (TA 240 S, Gould, Valley View, OH) was placed through the left atrium into the left ventricle, and adjusted to a left ventricular end-diastolic pressure (LVEDP) of 10 mm Hg. The LVEDP, the left ventricular developed pressure (LVDP, mm Hg) and the heart rate (HR, beats $\cdot$ min^{-1}) were continuously recorded.

Coronary flow (CF, mL $\cdot$ min^{-1}) was measured by timed collection of the coronary effluents. The incidence and duration of ventricular arrhythmias were measured during the reperfusion period. Only hearts with LVDP between 60 and 130 mm Hg, CF 9 to 18 mL $\cdot$ min^{-1}, and HR 240 to 360 beats $\cdot$ min^{-1} at the end of stabilization were included.

Myocardial Microdialysis

The technique of cardiac microdialysis was used to sample interstitial fluid (ISF) adenosine and NO metabolites during experimentation. A single 10-mm polycarbonate membrane microdialysis probe (CMA 20, Phymep, Solna, Sweden) was implanted in the left ventricular wall. The probe was perfused with a Ringer buffer containing (in mmol $\cdot$ L^{-1}): NaCl 140; KCl 4; $CaCl_2$ 1.26; $MgCl_2$ 1.15 at a constant flow rate of 1.33 mL $\cdot$ min^{-1} by a micropump (CMA 100, Phymep, Sweden). A 20-μL sample of ISF was collected every 15 minutes during the experiment by a collector (CMA 140, Phymep, Sweden) and stored at −20°C for later analysis.

Experimental Protocol

Rats were randomly divided into two groups, control (n = 10) and L-arginine (n = 10), and were injected, respectively, with saline buffer (3.30 mL $\cdot$ kg^{-1}) and L-arginine (500 mg $\cdot$ kg^{-1}) IP 24 hours before experimentation. Before heart excision a 1 mL blood sample was collected in the descending aorta. After centrifugation the serum was stored at −20°C for later anlaysis of cTnI and cGMP. After aortic cannulation, the microdialysis probe was inserted into the left ventricle wall.

The hearts were then stabilized for 15 minutes before starting the experimentation. All hearts were submitted to the same protocol: after 15 minutes stabilization by K-H perfusate, hearts were arrested by perfusion of 10 mL St Thomas' II cardioplegic solution (ST) at 4°C into the aortic root. All hearts were subjected to global ischemia at 20°C for 60 minutes. At the end of the global ischemia they were reperfused for 120 minutes by the K-H at 37°C at the same pressure (80 mm Hg). LVDP (LVSP-LVEDP), HR, and CF were measured in both groups before ST perfusion and 30, 60, 90, and 120 minutes after the start of reperfusion. The coronary effluents were collected at the above times and stored at −20°C for later analysis (see Biochemical Measurement).

Biochemical Measurement

Nitrite, a NO metabolite, was used as an index of NO release and was assayed in the ISF samples. Microdialysis ISF samples were also assayed for interstitial adenosine release. A 10-μL ISF sample assayed for adenosine using an automated high-performance liquid chromatography (HPLC) system (717 Plus autosampler, Waters, Millford, MA). Adenosine was separated using an Ultraspher ODS column (4.6 × 150 mm-5 μm, Beckmann, Fullerton, CA), with the mobile phase consisting of 12% (volume) methanol and 100 mmol $\cdot$ L^{-1} KH_2PO_4. Flow rate was set at 1 mL $\cdot$ min^{-1} using a pumping system (Waters 590, Waters, Millford). Eluent absorbance was continously monitored at 254 nm using an ultraviolet detector (Waters 486, Waters, Millford). The absorbance peak of adenosine was identified and quantified by comparing retention times and peak area to a known external standard of adenosine at a concentration of 1 μmol $\cdot$ L^{-1}.

A 5-μL sample of collected ISF was injected into a chemiluminescence nitric oxide analyzer (Sievers 280, Boulder, CO). Adenosine, NO metabolites, cTnI, and cGMP were assayed in coronary effluents. Cardiac TnI and cGMP were assayed in serum samples. cTnI measurement was performed by an Access automated immunoassay system (Beckman, Fullerton). cGMP was assayed using a radioimmunoassay diagnostic kit from Amersham International.

Electron Microscopy

At the end of reperfusion period, tissue samples (4 per heart) were taken from the left ventricle wall in all hearts. Then samples were cut into 1 mm^3, fixed in glutaraldehyde buffer for 2 hours at 4°C, embedded in epoxy resine, and examined in a double-blind study in an electron microscope (Jeol, 1213).

Chemicals

St Thomas' II cardioplegic solution was obtained from Aguettant Laboratory (Lyon, France). L-arginine was purchased from Sigma Chemicals Ltd (Lyon, France), and dissolved in saline buffer.

Statistics

Statistical comparisons were performed by a repeated measures analysis of variance (ANOVA). When significant differents were found a Bonferroni test was employed for pairwise comparisons. Differences were considered to be statistically significant when a p value was less than 0.05.

Results

Exclusion

A total of 30 rats were used for this study. Of the 15 rats assigned to the control, 1 was excluded because of ventricular fibrillation during stabilization and 2 because of poor hemodynamic factors and 2 because of technical problems. Of the 15 rats assigned to the L-arginine group, 2 were excluded because of poor hemodynamic factors and 3 because of technical problems.

Table 1. Myocardial Hemodynamic Parameters

		Reperfusion			
Before Ischemia		30 Minutes	60 Minutes	90 Minutes	120 Minutes
Heart rate					
Control (n = 10)	250 ± 10 (beats min^{-1})	91 ± 5	80 ± 7[a]	79 ± 5[a]	78 ± 4[a]
L-arginine (n = 10)	254 ± 16 (beats min^{-1})	98 ± 7	96 ± 8	97 ± 8	99 ± 7[b]
Left ventricular developed pressure					
Control (n = 10)	75 ± 3 (mm Hg)	65 ± 3[a]	60 ± 2[a]	58 ± 5[a]	60 ± 4[a]
L-arginine (n = 10)	99 ± 4[b] (mm Hg)	70 ± 4[a]	63 ± 3[a]	59 ± 3[a]	58 ± 4[a]
Coronary flow					
Control (n = 10)	10.3 ± 0.8 (mL min^{-1})	66 ± 4[a]	58 ± 4[a]	52 ± 4[a]	51 ± 4[a]
L-arginine (n = 10)	16.5 ± 2.1[b] (mL min^{-1})	66 ± 5[a]	63 ± 6[a]	56 ± 6[a]	54 ± 6[a]

Hemodynamic variables before ischemia (means ± SEM) and reperfusion period expressed as percentage of before ischemia values.

[a] $p < 0.05$ compared with before ischemia value. [b] $p < 0.05$ compared with control at the same time point.

Hemodynamic Data

Table 1 lists the hemodynamic variables measured in both groups before ischemia and during the reperfusion period. The hemodynamic variables of the reperfusion period subsequent to 60 minutes of ischemia are expressed as percentage of before ischemia values. There were no differences between the groups for HR after stabilization and during the reperfusion period except after 2 hours of reperfusion where HR was significantly higher in the L-arginine than in the control group. The percentage of HR recovery was improved in the L-arginine group. The LVDP and the CF were significantly higher in the L-arginine group before ischemia and during reperfusion even if the percentage of recovery was the same in the both groups. Throughout the reperfusion period, no arrhythmias occurred in either group.

Biochemical Assay

To avoid the influence of coronary flow on biochemical marker concentration in the coronary effluents, the corresponding values were multiplied by the CF to obtain biochemical marker flow expressed as $mmol \cdot min^{-1}$. As the flow rate of ISF was constant, values of ISF levels were expressed as concentration ($\mu mol \cdot L^{-1}$).

NO Metabolites Release Assay

Before experimentation, K-H and Ringer buffer were assayed for nitrite and nitrate. All solutions were nitrite and nitrate free.

In the ISF, mean NO metabolite levels were the same in both groups before ischemia (3.8 ± 0.7 versus 4.5 ± 0.9 $\mu mol \cdot L^{-1}$, p = not significant). When comparing NO metabolites before and immediately after ischemia, this value was significantly higher just after ischemia in the control group (8.2 ± 0.3 versus 3.8 ± 0.7 $\mu mol \cdot L^{-1}$, $p < 0.05$) and steady in the L-arginine group (2.4 ± 0.6 versus 4.5 ± 0.9 $\mu mol \cdot L^{-1}$, p = NS). In the control group, NO metabolites peaked 15 minutes after the onset of reperfusion at 12.0 ± 0.5 $\mu mol \cdot L^{-1}$ and decreased progressively to reach the same levels as in the L-arginine group (1.7 ± 0.5 $\mu mol \cdot L^{-1}$) which were steady throughout the experimentation (data not shown).

In the coronary effluents during reperfusion period, there were no differences for NO metabolite release between control and L-arginine groups. NO metabolite released in the coronary effluents remained steady during reperfusion (data not shown).

Adenosine Release Assay

Mean adenosine concentration in the ISF after 15 minutes stabilization, was 7.6 ± 1.3 $\mu mol \cdot L^{-1}$ in the control and 9.6 ± 2.3 $\mu mol \cdot L^{-1}$ in the L-arginine group (p = NS) and then decreased rapidly in both groups to reach 0.5 ± 0.02 $\mu mol \cdot L^{-1}$. Global ischemia as well as the reperfusion period did not modify the kinetics of adenosine levels in the ISF (data not shown).

In the coronary effluents before ischemia, the adenosine release was significantly higher in the L-arginine when compared with the control group (55.6 ± 15 versus 6.3 ± 3 $nmol \cdot min^{-1}$; $p < 0.001$). During global ischemia, adenosine release increased in the L-arginine group when compared with the control (92.2 ± 25 versus 11.5 ± 4 $nmol \cdot min^{-1}$; $p < 0.001$). During the reperfusion period, the adenosine release was almost steady in both groups but remained significantly higher in the L-arginine than in the control group (Fig 1).

cGMP Release Assay

The serum concentration of cGMP before heart excision was not different in L-arginine and control groups (8.0 ± 0.5 versus 6.9 ± 0.9 $nmol \cdot L^{-1}$; p = NS). Ischemia and reperfusion did not modify cGMP release in the coronary effluents. There were no differences of cGMP release between both groups (data not shown).

cTnI Release Assay

The serum concentration of cTnI drawn before heart excision was significantly higher in the L-arginine than in the control group (0.037 ± 0.01 versus 0.02 ± 0.05 $\mu g \cdot L^{-1}$; $p < 0.05$; Fig 2A). Before ischemia, cTnI release in coronary effluents was higher in the control group than in the L-arginine group (2.2 ± 0.5 versus 0.6 ± 0.2 $ng \cdot min^{-1}$, $p < 0.001$). During the reperfusion period, the level of cTnI release, even if decreasing, remained significantly higher in the control than in the L-arginine group (Fig 2B).

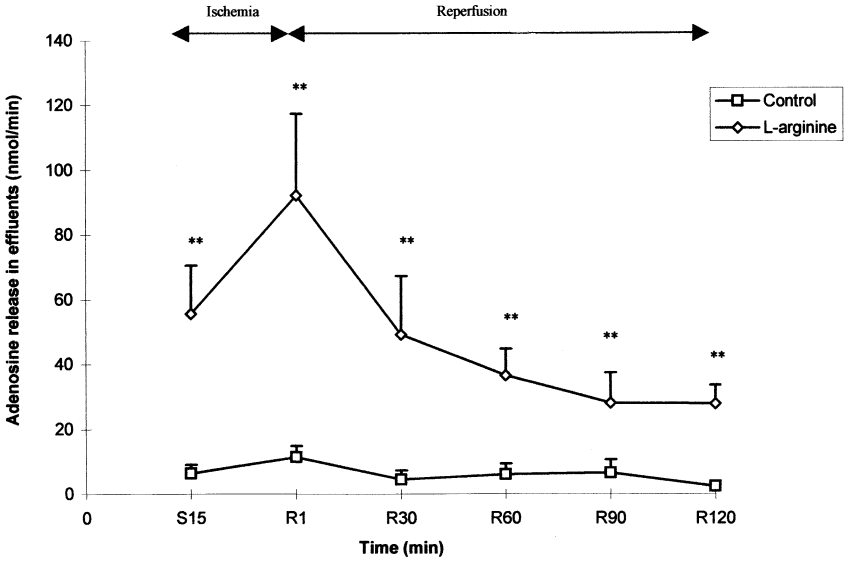

*Fig 1. Changes in adenosine in heart. Adenosine release in coronary effluents was measured 15 minutes after stabilization (S15) and at reperfusion 1 minute (R1), 30 minutes (R30), 60 minutes (R60), 90 minutes (R90), and 120 minutes (R120). L-arginine group compared with control: **p < 0.001 versus control. Data are mean ± SEM.*

Electron Microscopic Observations

In the control group mitochondrias were not damaged (Fig 3A). In the L-arginine group, the mitochondrias were severely swollen and the cristae were disrupted (Fig 3B). These injuries were considered to be reversibles. In both groups, there was no damage to the sarcomeres and endothelial cells.

Comment

Ischemia-reperfusion is known to injure endothelial cells and consequently to impair vasodilatory response by a decrease in NO production [2]. Various procedures of NO production such as administration of NO, L-arginine and NO donor agents have been shown to protect the myocardium against ischemia-reperfusion injury. The role of exogenous L-arginine to decrease ischemia-reperfusion damage and improve myocardial function has been shown in regional or global ischemia. In cardiac surgery, L-arginine has been shown to be cardioprotective when added to cardioplegic solution or given during reperfusion [3–5, 7]. The effect of L-arginine when administered IP 24 hours before the ischemic injury is unknown.

The present study showed that the intraperitoneal administration of L-arginine before ischemia; (1) induced reversible damage to mitochondrias; (2) did not modifiy the NO metabolites and cGMP kinetic released in reperfusion; (3) increased the adenosine and decreased the cTnI release during reperfusion period; and (4) improved the hemodynamic variables during reperfusion. Ischemia affected identically the hemodynamic variables as shown by the similar percentage of recovery in both groups. As LVDP and CF were significantly higher in the L-arginine group before ischemia, they remained so throughout the experimentation.

Cardiac troponin I has been shown to be a specific marker of myocardial cell ischemia and injury [8, 9]. The pretreatment with L-arginine induced a higher release of cTnI in serum before heart excision which revealed, as well as mitochondria damage, the deleterous effects of L-arginine through NO generation on myocardium before ischemia. Abe and colleagues [11] have shown that generated endogenous NO and NO-derived species could damage mitochondria and cardiac cells during postischemic reperfusion. Conversely to Engelmann and associates [12], our study did not show an increase in cGMP induced by L-arginine. Contrary to studies using L-arginine in cardioplegic solution or during the reperfusion period, in our study the NO metabolite release level was the same during ischemia and reperfusion [13, 14]. The timing of L-arginine injection, its delivery route as well as its dose may be an explanation of this discrepancy [6, 12].

Adenosine is produced from ATP by the S-adenosylhomocysteine (SAH) catabolism or from AMP by ecto-5′-nucleotidase [15]. Adenosine is known to have a cardioprotective action in the ischemia-reperfusion myocardium when added to cardioplegic solution [16, 17] as well as a vasodilatory effect dependent of the amount released [18]. Our results showed that the pretreatment with L-arginine was associated with a higher release of adenosine in preischemia as well as during the reperfusion period. The higher CF and LVDP observed in the L-arginine group before ischemia could be explained by the effect of adenosine release on coronary flow.

In the L-arginine group swollen mitochondrias corresponding to reversible lesions were found. There was no irreversible damage to the myocyte organization in both groups. Mitochondria damage decreases ATP synthetisation and increases ATP breakdown, which increases adenosine concentration within myocytes.

It can be hypothesized that mitochondria damage is due to L-arginine and occurs during the 24 hours between L-arginine administration and preischemia period as shown by a higher release of adenosine in the L-

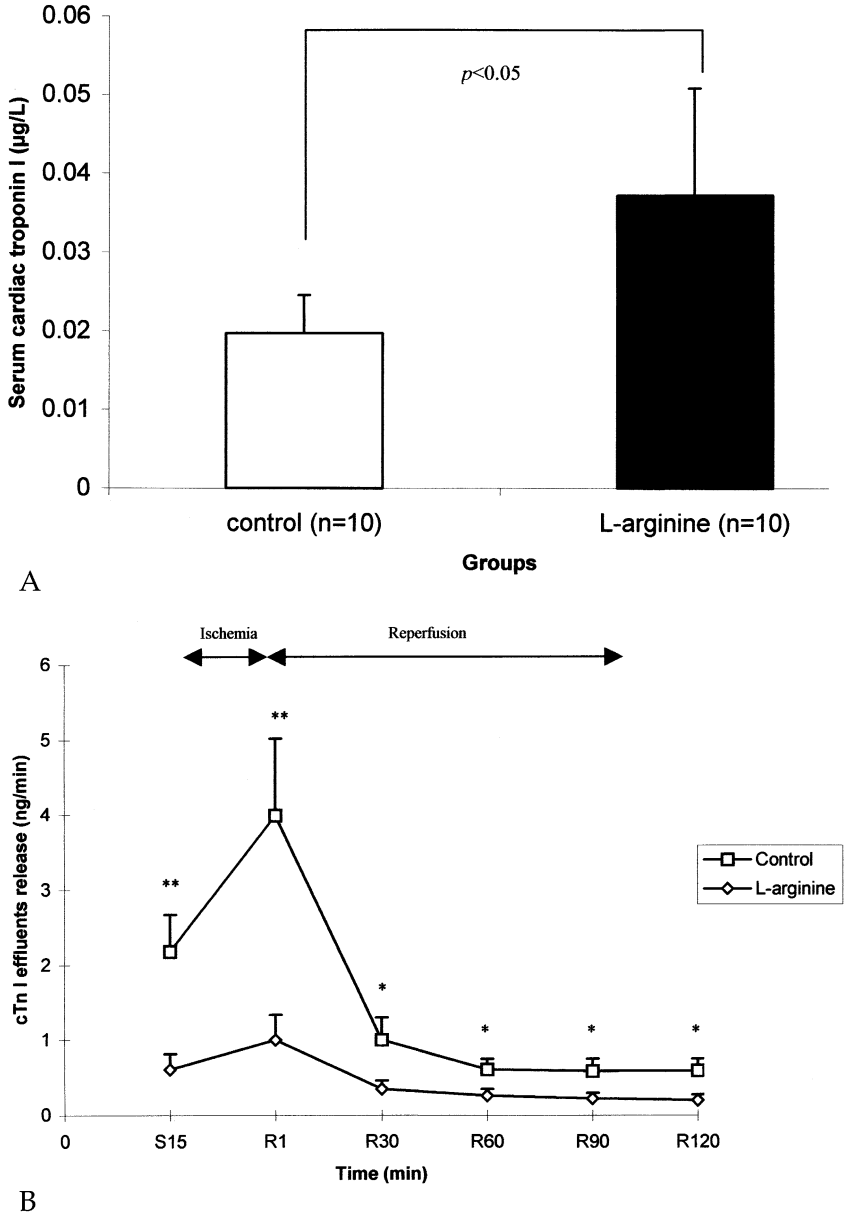

*Fig 2. Changes in myocardial cardiac troponin I (cTnI). (A) Bar graph of serum cTnI concentration obtained before heart extraction. In the L-arginine group there was a higher release of cTnI than in the control: $p < 0.05$. (B) Global ischemia increased the release of cTnI in coronary effluents in both groups. The levels of cTnI remained lower in the L-arginine group than in the control: **$p < 0.001$ versus control, *$p < 0.05$ versus control. The sampling times and abbreviations are the same as in Figure 1. Data are mean ± SEM.*

arginine group in the samples drawn before ischemia. Ischemia by itself also increased the ATP breakdown which increased the adenosine concentration. Therefore, there was a cumulative effect on adenosine concentration due to pretreatment by L-arginine and ischemia.

During reperfusion a washout of accumulated adenosine occurred explaining the higher release of adenosine in the L-arginine group before ischemia and during the reperfusion period. These observations suggest that the pretreatment with L-arginine induced a higher adenosine release before ischemia and during reperfusion and a lower release of cTnI during the same periods. This can be explained by the role of adenosine in the decrease of myocardial cell injury. This is in agreement with studies showing the vasodilatory effect of adenosine [18] and its contribution to myocardial protection after cardioplegia ischemic [19, 20]. Conversely, as shown by Belhomme and coworkers [21] the administration of exogenous adenosine to coronary artery bypass grafting patients did not influence the cTnI release.

In the present experiment, the ISF concentrations of adenosine and NO metabolites were used to estimate the interstitial concentration of adenosine and the NO metabolite release. Caution must be exercised in the extrapolation of ISF to the interstitial concentration.

In conclusion, the cardioprotective effect of L-arginine administration in cardioplegia solution or during reperfusion was demonstrated. Our data suggest that the administration of L-arginine 24 hours before cardioplegic arrest induces reversible damage to heart mitochondrias. The myocardial injury was also assessed by the higher cTnI concentration in the blood sample drawn before heart excision. This initial aggression by L-arginine administration seems to protect the myocardium from the subsequent

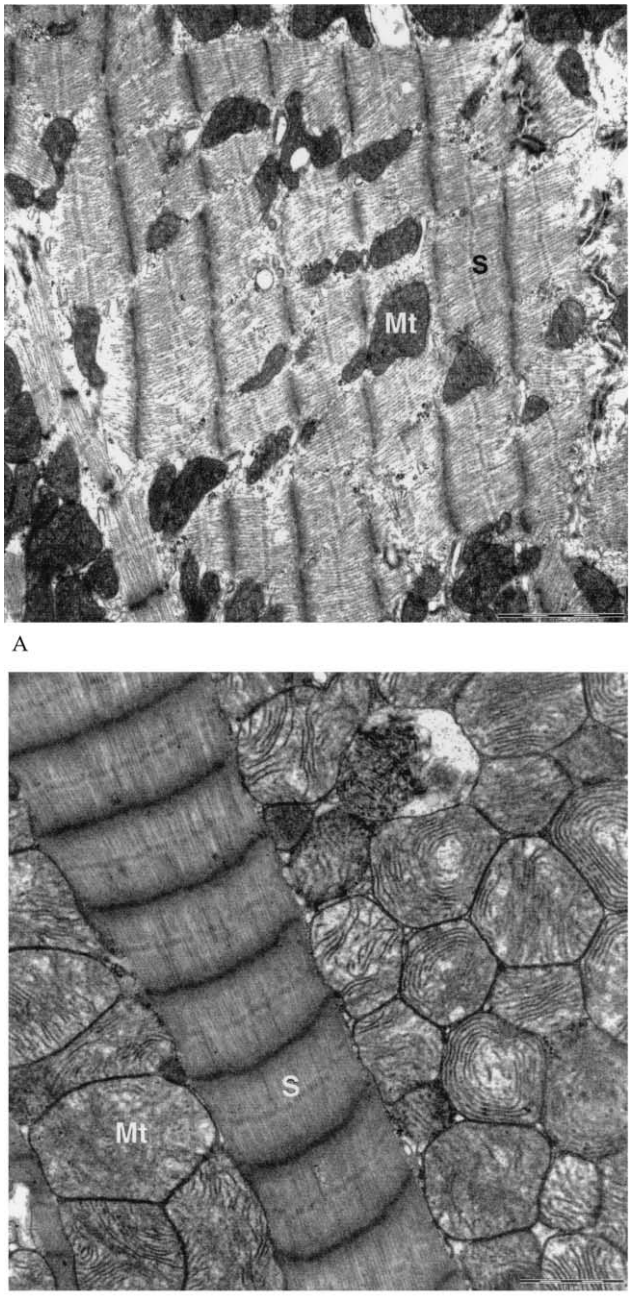

Fig 3. Electron microscopy of the heart mitochondrias in the left ventricle wall. (A) In the control group, there was no damage to mitochondrias (Mt) *and sarcomeres* (S). *(B) In the L-arginine group, the mitochondrias were edematous and the cristae were disrupted, but sarcomeres and endothelial cells were not damaged.* (bars = 1 μm.)

ischemia-reperfusion injury as assessed by the lower cTnI release in coronary effluents in the L-arginine group during reperfusion. In this way, L-arginine administered intraperitoneally 24 hours before cardioplegic arrest seems to protect myocardium against ischemia-reperfusion injury. These findings could be useful in clinical practice particularly in cardiac surgery. Before a clinical application, the specific mechanisms underlying pharmacologic protection by L-arginine must be explicit.

References

1. Moncada S, Higgs A. The L-arginine nitric oxide pathway. N Engl J Med 1993;329:2002–12.
2. Lefer AM, Tsao PS, Lefer DJ, Ma XL. Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. FASEB J 1991;5:2029–34.
3. Carrier M, Khalil A, Tourigny A, Solymoss BC, Pelletier LC. Effect of L-arginine on metabolic recovery of the ischemic myocardium. Ann Thorac Surg 1996;61:1651–7.
4. Izhar U, Schwalb H, Borman JB, Merin G. Cardioprotective effect of L-arginine in myocardial ischemia and reperfusion in an isolated working rat heart model. J Cardiovasc Surg 1998;39:321–9.
5. Hayashida N, Tomoeda H, Oda T, et al. Effects of supplemental L-arginine during warm blood cardioplegia. Ann Thorac Cardiovasc Surg 2000;6:27–33.
6. Noeh FM, Wenzel A, Harris N, et al. The effects of L-arginine administration on the levels of arginine, other amino acids and related amino compounds in the plasma, heart, aorta, vena cava, bronchi and pancreas of the rat. Life Sci 1996;58:131–6.
7. Sato H, Zhao ZQ, McGee DS, Williams MW, Hammon JW Jr, Vinten-Johansen J. Supplemental L-arginine during cardioplegic arrest and reperfusion avoids regional postischemic injury. J Thorac Cardiovasc Surg 1995;110:302–14.
8. Etievent JP, Chocron S, Toubin G, et al. Use of cardiac troponin I as a marker of perioperative myocardial ischemia. Ann Thorac Surg 1995;59:1192–4.
9. Chocron S, Alwan K, Toubin G, et al. Crystalloid cardioplegia route of delivery and cardiac troponin I release. Ann Thorac Surg 1996;59:1192–4.
10. Brown GC, Borutaite V. Nitric oxide, cytochrome c and mitochondria. Biochem Soc Symp 1999;66:17–25.
11. Abe K, Hayashi N, Terada H. Effect of endogenous nitric oxide on energy metabolism of rat heart mitochondria during ischemia and reperfusion. Free Radic Biol Med 1996;26:379–87.
12. Engelman DT, Watanabe M, Maulik N, et al. Critical timing of nitric oxide supplementation in cardioplegic arrest and reperfusion. Circulation 1996;96:II407–11.
13. Engelman DT, Watanabe M, Engelmann RM, et al. Constitutive nitric oxide release is impaired after ischemia and reperfusion. J Thorac Cardiovasc Surg 1995;110:1047–53.
14. Engelman DT, Watanabe M, Maulik N, et al. L-arginine reduces endothelial inflammation, and myocardial stunning during ischemia/reperfusion. Ann Thorac Surg 1995;60: 1275–81.
15. Ohisalo JJ. Regulatory functions of adenosine. Med Biol 1987; 65:181–91.
16. Schubert T, Vetter H, Owen P, Reichart B, Opie LH. Adenosine cardioplegia. Adenosine versus potassium cardioplegia: effects on cardiac arrest and postischemic recovery in the isolated rat heart. J Thorac Cardiovasc Surg 1989;98: 1057–65.
17. Nawas SI, Schwertz DW, Beck JM, Fogelson BG, Law WR. Mechanisms of myocardial protection by adenosine-supplemented cardioplegia: differential response of calcium-independent protein kinase C isoenzymes. J Surg Res 2000;89:163–8.
18. Ely SW, Matherne GP, Coleman SD, Berne RM. Inhibition of adenosine metabolism increases myocardial interstitial adenosine concentrations and coronary flow. J Mol Cell Cardiol 1992;24:1321–32.
19. Fogelson BG, Nawas SI, Law WR. Mechanisms of myocardial protection in adenosine-supplemented cardioplegia: myofilament and metabolic responses. J Thorac Cardiovasc Surg 2000;119:601–9.
20. Nishida T, Masuda M, Kanegae Y, et al. The differential cardioprotective effects of nucleoside transport inhibitor on moderate and deep hypothermic ischemia with cold cardioplegia. Surg Today 2000;30:805–10.
21. Belhomme D, Peynet J, Florens E, Tibourtine O, Kitakaze M, Menasché P. Is adenosine preconditioning truly cardioprotective in coronary artery bypass surgery? Ann Thorac Surg 2000;70:590–4.