The Effects of L-Arginine Administration on Guinea Pig Heart Following Ischemia and Reperfusion

L-ARGİNİN UYGULAMASININ İSKEMİ VE REPERFÜZYON SONRASINDA KOBAY KALBİNE ETKİSİ

Volkan SİNCÎ*, Sedat KALAYCIOĞLU*, Serdar GÜNAYDIN*, Yıldırım İMREN*, Eser ÖZ**, Halim SONCUL*, Levent GÖKGÖZ*, Ali ERSÖZ*

* Dept. of Cardiovascular Surgery, Medical School of Gazi University,
** Dept. of Physiology, Medical School of Gazi University, Ankara, TURKEY

Summary

L-arginine has been demonstrated to stimulate the release of nitric oxide, but its effects on recovery and the optimal administration time have been controversial.

The hearts from 20 controls and 40 study group guinea pigs were mounted on a Langendorff perfusion apparatus and were perfused by gassed Krebs-Henseleit solution (KH). The hearts were then allocated into 4 study groups: First 2 groups, under hypothermia, exposed to 15 min. ischemia and either received preischemic L-arginine and only KH during reperfusion (Group III) or preischemic and postischemic L-arginine (Group IV); the other 2 groups under normothermia, exposed to 15 min. ischemia and either received preischemic L-arginine and only KH for reperfusion (Group V) or preischemic and postischemic L-arginine (Group VI).

Percentage recovery of heart rate, developed pressure and dp/dt were significantly higher for Groups III-IV and V-VI than the controls ($p<0.05$). Malondialdehyde levels were significantly lower for Groups III-IV and V-VI than the controls. Hypothermic control levels were significantly better than normothermic controls ($p<0.05$). NO'_2 and glutathione levels were significantly higher for Groups III-IV and V-VI than the controls. Hypothermic control levels were significantly better than normothermic controls ($p<0.05$).

Our study demonstrated no difference in the timing of administration of L-arginine either before ischemia or both in preischemia and reperfusion.

Key Words: Nitric Oxide, Coronary circulation, Myocardial reperfusion injury

T Klin J Med Res 1999, 17:104-110

Özet

L-arginin'in nitrik oksit saliminim artırdığı gösterilmiş olmakla birlikte, kalbin derlenmesi üzerindeki etkileri ve optimal zamanlama konusundaki tartışmalar devam etmektedir.

20'si kontrol ve 40'ı çalışma grubundan alınan kobay kalpleri Langendorff modelinde çalışılarak Krebs-Henseleit (KH) çözeltisi ile perfüze edilmiştir. Daha sonra kalpler 4 çalışma grubuna ayrılmıştır: İlk iki grup hipotermide 15 dakikalık iskemi zamanı ile iskemi öncesi L-arginine ve sonrasında KH (Grup III); veya iskemi öncesi ve sonrası L-arginine (Grup IV) ile perfüze edilmiş; normotermide çalışılan diğer iki grupta ise 15 dakikalık iskemi uygulanmış, iskemi öncesi L-arginine ve sonrasında KH (Grup V); veya iskemi öncesi ve sonrası L-arginine (Grup VI) ile perfüze edilmiştir.

Kalp hızı derlenme yüzdesi, dp, dp/dt Grup III-IV ve V-VI için kontrol gruplarından anlamlı olarak daha iyiydi ($p<0.05$). Malondialdehit (MDA) düzeyleri ise çalışma gruplarında kontrollerden anlamlı olarak daha düşüktü. Hipotermik kontrol grubu normotermik kontrolden anlamlı olarak daha iyiydi.($p<0.05$). NO_2^- ve glutatyon düzeyleri çalışma gruplarında kontrollerden anlamlı olarak daha yüksekti. Hipotermik kontrol grubu normotermik kontrolden anlamlı olarak daha iyiydi.($p<0.05$).

Çalışmamız L-arginin 'in iskemiden önce veya hem önce hem sonra uygulanmasının arasında bir fark olmadığım göstermiştir.

Anahtar Kelimeler: Nitrik oksit, Koroner dolaşım, Miyokardiyal reperfüzyon hasarı

TKlin Araştırma 1999, 17:104-110

Myocardial ischemia/ reperfusion (I/R) have demonstrated damage to endothelium, impaired production and release of vasoactive substances and marked alterations in endothelium-dependent relaxation of the coronary vasculature. Release of nitric oxide (NO) from coronary endothelial cells has been found to be impaired and contributed to the vulnerability of the coronary circulation, thrombus formation and vasospasm(l). However, the mechanism of action and results of exogenous NO

Received: May 11, 1999

Correspondence: Serdar GÜNAYDIN
Emek Mah. 60.sok No: 138/26
06510 Ankara, TURKEY

supplementation are still controversial. Several experimental studies have found that NO has a cardioprotective effect in I/R injury and some others suggested that NO exacerbates I/R injury and that NO synthase inhibitors may protect myocardium.

This study was designed to test the hypothesis that if exogenous administration of L-arginine, a precursor of nitric oxide, in hearts subjected to global ischemia, cardioplegic arrest and reperfusion may improve I/R injury by stimulation of NO production or not.

Materials and Methods

Hearts were obtained from male guinea pigs (N=60, 10 for each group) weighing 300-450 grams. A11 animals received human care in compliance with the 'Principles of Laboratory Animal Care' formulated by the National Society for Medical Research and the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratoiy Animal Resources and published by the National Institute of Health (NIH publication No.86-23, revised 1985). University Research Ethics Committee approval was also obtained.

The animals were anesthetized by thiopental 20 mg/kg and given 200 units of heparin into femoral vein. The hearts (1.8-2.5 gr) were rapidly removed and cannulated via aortic root and then mounted on a modified Langendorff perfusion apparatus and perfused by a gassed (Oxygen 95%, Carbondioxide 5%) Krebs-Henseleit solution (KH) at a rate of 10 ml/min at 37°C. The composition of the solution was $NAHC0_3$: 25mMol/L, NaCl:118mMol/L, KH_2P0_4:1.2 mMol/L, KCL4.8 mMol/L, $MgS0_4$:1.2 mMol/L, $CaCl_2$:1.2 mMol/L and glucose: 11.1 mMol/L.

Following a 10-min stabilization period under KH perfusion, the animals were allocated into 6 groups, 10 animals in each:

Group I was a control group for hypothermic ischemia: hearts underwent hypothermic (20°C) ischemic arrest for 15 min. by stopping Krebs-Henseleit solution and administration of St. Thomas cardioplegia and then reperfused by Krebs-Henseleit solution.

Group II was a control group for normothermic ischemia: hearts underwent normothermic ischemic arrest for 15 min. by stopping Krebs-Henseleit solution and administration of St. Thomas cardioplegia and then reperfused by Krebs-Henseleit solution.

In Group III, hearts underwent hypothermic (20°C) ischemic arrest for 15 min. by stopping Krebs-Henseleit solution and administration of St. Thomas cardioplegia supplemented by lOmMol/L L- arginine (Sigma Chemical Co., St.Louis, MO). Hearts were then reperfused by only Krebs-Henseleit solution for 15 min.

In Group IV, hearts underwent hypothermic (20°C) ischemic arrest for 15 min. by stopping Krebs-Henseleit solution and administration of St. Thomas cardioplegia supplemented by 10mMol/L L- arginine. Hearts were then reperfused by Krebs-Henseleit solution supplemented by 10 mMol/L L-arginine for 15 min.

In Group V, hearts underwent normothermic ischemic arrest for 15 min. by stopping Krebs-Henseleit solution and administration of St. Thomas cardioplegia supplemented by lOmMol/L L- arginine. Hearts were then reperfused by only Krebs-Henseleit solution for 15 min.

In Group VI, hearts underwent normothermic ischemic arrest for 15 min. by stopping Krebs-Henseleit solution and administration of St. Thomas cardioplegia supplemented by lOmMol/L L- arginine. Hearts were then reperfused by Krebs-Henseleit solution supplemented by 10 mMol/L L-arginine for 15 min.

For hemodynamic evaluation, the left ventricular maximum developed pressure (Dp-defined as peak aortic systolic minus end-diastolic pressure), and the first derivative dp/dt were measured using intraventricular baloon before ischemia (baseline) and at the end of reperfusion. Direct measurements of heart rate (% change), developed pressure (Dp) and dp/dt (% change) were determined and assessed. Pressures were measured by a Datascope 2001A monitor.

Coronary perfusion was collected and preischemic/postischemic glutathione, malondialdehyde (MDA) and $NO"_2$ levels were measured.

All hearts were then preserved in a liquid nitrogen tank at -196°C and later tissue $NO"_2$, MDA and gluthatione levels were also calculated.

Nitrites ($NO"_2$), were obtained as the last products of NO metabolism. The amount of total nitrite (mmol) was determined by a modification of the procedure described by Braman and Hendrix (2), using the purge system of a Sievers Instruments Model 280A NO analyzer. Besides NO levels, in order to determine tissue damage, MDA (nmol/ml) and glutathione (nmol/ml) were measured at the preischemic period and after reperfusion in the coronary perfusate. Tissue MDA (nmol/g tissue) and tissue glutathione (|xmol/g tissue) levels were also calculated (3,4).

The results were presented as mean and standard error of mean. Overall significance of differences between groups were determined by unpaired t test using Microsoft Excel 97 PC program.

Results

Results are given in Figures 1-4.

In Group I (control for hypothermic arrest), the mean percentage change of the heart rate was 78.6±8% /min of the baseline, the mean percentage recovery of Dp was 75.3±8% and the percentage recovery of the dp/dt was 55.4±9%.

In Group II (control for normothermic arrest), the mean percentage change of the heart rate was 69.6±7% /min of the baseline, the mean percentage recovery of Dp was 63.2±8% and the percentage recovery of dp/dt was 42.3±9%.

However, we obtained a well- documented improvement in the hemodynamic parameters within the study groups.

In Group III, (hypothermic, preischemic L-arginine supplementation and postischemic KH only), the mean percentage change of the heart rate was 105.7.6±9% /rnin of the baseline, the mean percentage recovery of Dp was 114.6±8% and the percentage recovery of the dp/dt was 116.2±9%.

In Group IV, (hypothermic, preischemic and postischemic L-arginine supplementation), the mean percentage change of the heart rate was 108.6.6±8% /min of the baseline, the mean percentage recovery of Dp was 117.5±8% and the mean percentage recovery of dp/dt was 120.1±9%.

In Group V, (normothermic, preischemic L-arginine supplementation and postischemic KH only), the mean percentage change of the heart rate was 95.7.6±9% /min of the baseline, the mean percentage recovery of Dp was 98.7±8% and the mean percentage recovery of dp/dt was 96.2±9%.

v

In Group VI, (normothermic, preischemic and postischemic L-arginine supplementation), the mean percentage change of the heart rate was 97.4±8% /min of the baseline, the mean percentage recovery of Dp was 100.2±8% and the mean percentage recovery of dp/dt was 98.3±8%.

There were statistically significant differences between Groups III-IV and hypothermic control Group I with respect to mean percentage change of heart rate ($p<0.001$), mean percentage recovery of Dp ($p<0.0001$) and mean percentage recovery of dp/dt ($p<0.0001$).

There were no significant differences in any of the hemodynamic parameters between Groups III andlV.

Comparing Groups V-VI and normothermic control Group II, there were significant differences in respect of mean percentage change of heart rate ($p<0.001$), mean percentage recovery of Dp ($p<0.001$)and mean percentage recovery of dp/dt ($p<0.001$).

There were no significant differences in any of the hemodynamic parameters between Groups V and VI.

Also demonstrating the importance of the hypothermic situations for the preservation of hemodynamic measurement, there were significant differences in respect of mean percentage change of heart rate ($p<0.05$), mean percentage recovery of Dp ($p<0.05$) and mean percentage recovery of dp/dt ($p<0.01$) between Groups I and If.

Comparing the corresponding study groups (III-V and IV-VI) for the importance of the hypothermic/normothermic situations, there were significant differences in respect of mean percentage change of heart rate ($p<0.05$), mean percentage recovery of Dp ($p<0.05$) and mean percentage recovery of dp/dt ($p<0.05$) between Groups III and V as well as Groups IV and VI.

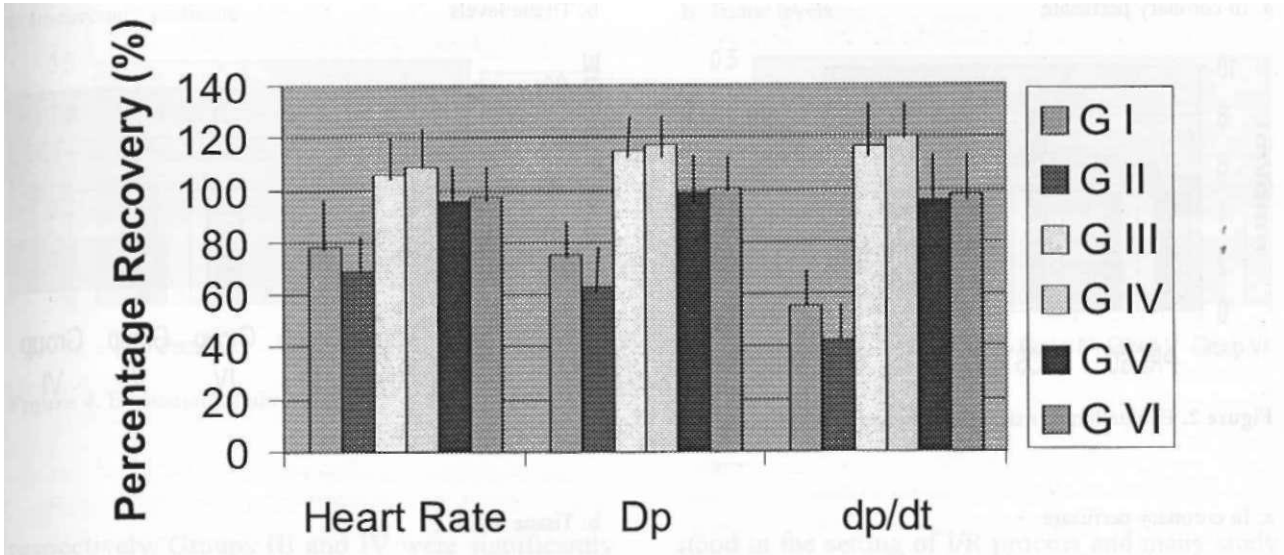

Figure 1. Hemodynamic evaluation of the groups. Dp: Developed pressure dp/dt: First derivative *:p≤0.001

In Group I, mean perfusate MDA levels were calculated to be 4.82±0.1 before ischemia and 6.2±0.1 after reperfusion. In Group II, mean perfusate MDA levels were calculated to be 4.72±0.1 before ischemia and 8.2±0.1 after reperfusion.

Mean perfusate MDA levels were calculated to be 4.85±0.1 before ischemia and 4.9±0.1 after reperfusion in Group III and 4.79±0.1 before ischemia and 4.88±0.1 after reperfusion in Group IV. For Group V, mean MDA perfusate levels were 4.83±0.1 before ischemia and5.2±0.1 after reperfusion. They were 4.84±0.1 before ischemia and 5.0±0.1 after reperfusion for Group VI.

Groups III and IV were significantly better than control (Group I) ($p<0.01$) and there was no significant difference between groups III and IV.

Groups V and VI were significantly better than control (Group II) ($p<O.0T$) and there was no significant difference between groups V and VI.

Hypothermic groups were significantly better than normothermic corresponding groups (Groups I-II, **m-v, iv-vi,,<o.o5).**

In Group I, mean perfusate glutathione levels were calculated to be 83.9±4 before ischemia and 70.4±6 after reperfusion.

In Group II, mean perfusate glutathione levels were 85.1±5 before ischemia and 61.3±5 after reperfusion.

Mean perfusate glutathione levels were 82.3±5 before ischemia and 82.2±0.1 after reperfusion in Group III and 86.1±4 before ischemia and 81.8±5 after reperfusion in Group IV. For Group V, mean glutathione perfusate levels were 84±5 before ischemia and 78±0.1 after reperfusion. They were 84.3±5 before ischemia and 77.5±6 after reperfusion for Group VI.

Groups III and IV were significantly better than control (Group I) ($p<0.01$) and there was no significant difference between groups III and IV.

Groups V and VI were significantly better than control (Group II) ($p<0.01$) and there was no significant difference between groups V and VI.

Hypothermic groups were significantly better than normothermic corresponding groups (Groups I-II, III-V, IV-VI, $p<0.05$).

In Group I, mean perfusate NO levels were calculated to be 0.14±0.0T ppb before ischemia and 0.03±0.0T ppb after reperfusion.

In Group II, mean perfusate NO levels were 0.17±0.01 ppb before ischemia and 0.008±0.002 ppb after reperfusion.

Mean perfusate NO levels were 0.16±0.0T ppb before ischemia and 0.39±0.03 after reperfusion in Group III and 0.19±0.01 before ischemia and 0.35±0.05 after reperfusion in Group IV. For Group

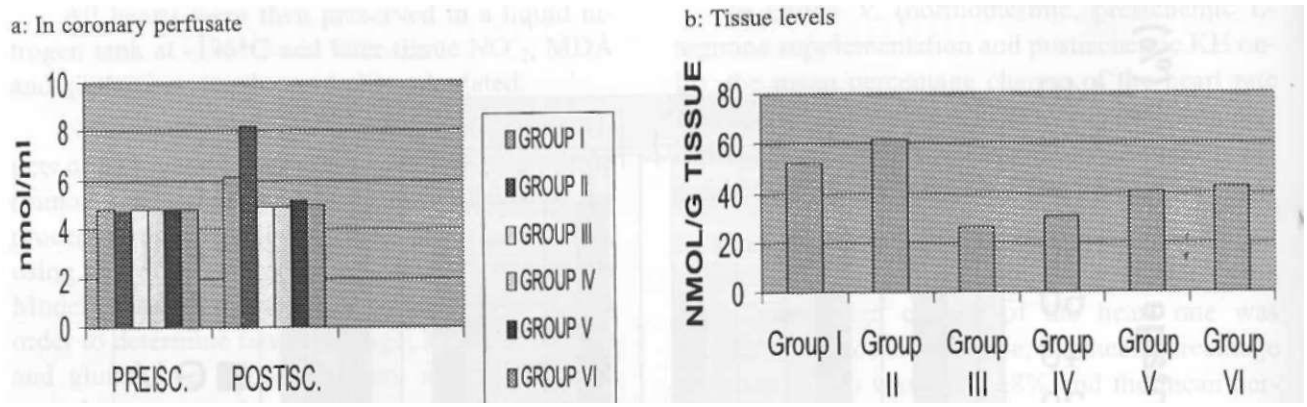

Figure 2. Evaluation of malondialdehyde (MDA) levels, *:p<0.01

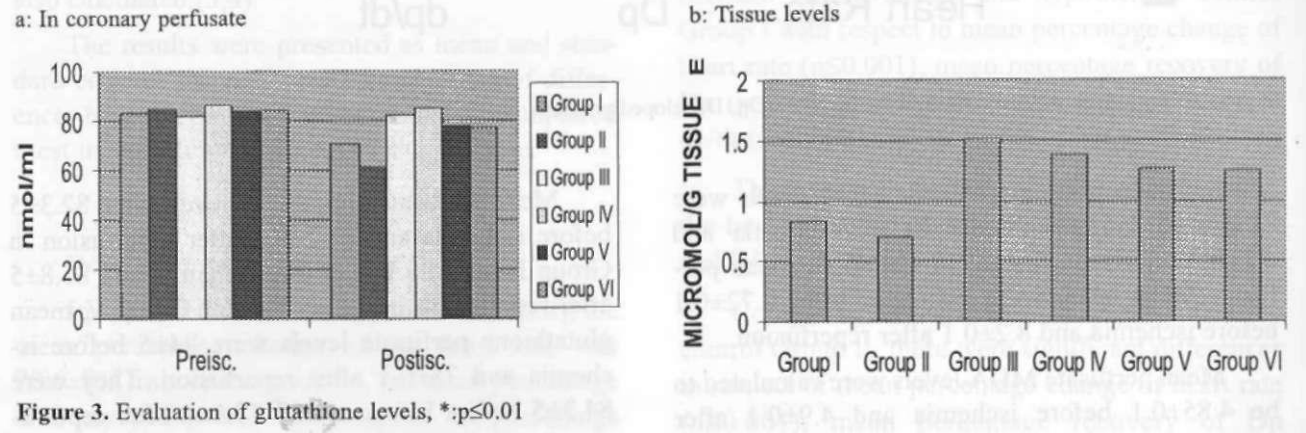

Figure 3. Evaluation of glutathione levels, *:p≤0.01

V, mean NO perfusate levels were 0.15±0.02 before ischemia and 0.25±0.04 after reperfusion. They were 0.12±0.0T before ischemia and 0.27±0.03 after reperfusion for Group VI.

Groups III and IV were significantly better than control (Group I) (p<0.01) and there was no significant difference between groups III and IV.

Groups V and VI were significantly better than control (Group II) (p<0.01) and there was no significant difference between groups V and VI.

Hypothermic groups were significantly better than normothermic corresponding groups (Groups I-II, III-V, IV-VI, p<0.05).

The RIA measurements in the preserved hearts were also evaluated.

In the hypothermic control group (Group I), mean tissue NO level was calculated to be 0.09±0.006 ppb. In Group II, it was 0.04±0.005 ppb.

Mean tissue NO levels were 0.41 ±0.01 ppb in Group III and 0.35±0.01 in Group IV. For Group V, mean NO tissue level was 0.27±0.02. It was 0.25±0.01 for Group VI. Groups III and IV were significantly better than control (Group I) (p<0.01).

There was no significant difference between . groups III and IV.

Groups V and VI were significantly better than control (Group II) (p<0.01) and there was no significant difference between groups V and VI.

Hypothermic groups were significantly better than normothermic corresponding groups (Groups I-II, III-V, **rv-vi, p<o.os).**

As tissue MDA levels concerned, in Group I, mean tissue level was 52.7±5. In Group If, it was 61±6. Mean tissue MDA levels were 26.2±3 in Group III and 30.1 ±3 in Group IV. For Group V and VI, mean tissue levels were 40.2±4 and 42.3±5,

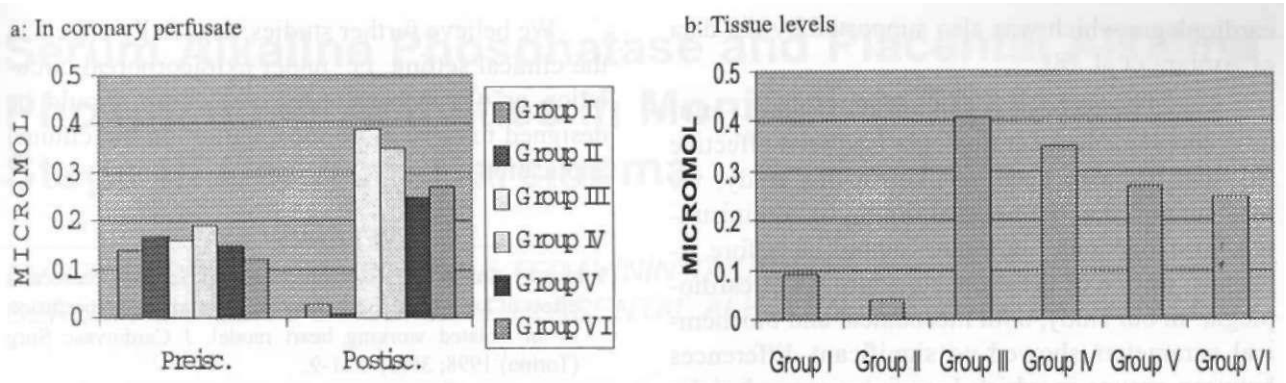

Figure 4. Evaluation of nitrite levels, *:p≤0.01

respectively. Groups III and IV were significantly better than controls (Group I) (p<0.001).There was no significant difference between groups III and IV.

Groups V and VI were significantly better than controls (Group II) (p<0.01) and there was no significant difference between groups V and VI.

Hypothermic groups were significantly better than normothermic corresponding groups (Groups I-II, III-V, IV-VI, p<0.01).

For tissue glutathione levels, in Group I, mean tissue level was 0.84±0.05. In Group II, it was 0.70±0.04. Mean tissue glutathione level was 1.51±0.07 in Group III and 1.38±0.05 in Group IV. For Group V, mean tissue level was 1.27±0.06. It was 1.25±0.05 for Group VI. Groups III and IV were significantly better than control (Group I) (p<0.001).

There was no significant difference between groups III and IV.

Groups V and VI were significantly better than controls (Group II) (p<0.01) and there was no significant difference between groups V and VI.

Hypothermic groups were significantly better than normothermic corresponding groups (Groups I-II, III-V, IV-VI, p<0.01).

Discussion

Basic amino acid L-Arginine has been known to have several biological actions including NO production. The real effects caused by L-arginine administration to heart has not been fairly understood in the setting of I/R process and many study designs had controversial data, especially about the critical timing and cardioprotective effects of L-arginine administration (5).

Several experimental studies have found that NO has a cardioprotective effect in I/R injury. In this regard, attempts have been made to supplement NO production exogenously during reperfusion, when endogenous NO release from endothelial cells may be diminished. In this sense, L-arginine has been shown to act beneficially in two possible mechanisms, either by blocking both neutrophil aggregation and neutrophil adherence or by scavenging oxygen-derived free-radicals in blood reperfused models and by direct vasodilation as well as reduced oxygen demand due to vasodilation induced hypotension in non-blood reperfused models (6).

Some other studies, therefore, have suggested that NO exacerbated I/R injury and that NO synthase inhibitors have acted as cardioprotective due to the prevention of peroxinitrite formation from NO and superoxide during reperfusion (6).

Since Engelman et al. also showed that L-arginine given during reperfusion was deleterious to optimal recovery of myocardial function in an I/R model (7), we have preferred to administer L-arginine only before ischemia and compared with other groups which was administered before ischemia and in reperfusion as well.

One of the other aspects in our study was that hypothermic groups were much better than normothermic groups. We showed that L-arginine administration should better be within hypothermic

cardioplegia which was also supported by the data of Amrani et al. (8).

Since many studies demonstrated that L-arginine supplemented cardioplegia has been effective (9), that has also been verified in this study; we may suggest that the optimal timing of administration of exogenous L-arginine should be before ischemia, as one of the probable additives of cardioplegia. In our study, both mechanical and biochemical parameters showed no significant differences between groups in which L-arginine was administered only before ischemia or in the preischemic as well as the reperfusion periods.

Because of the above-mentioned contradictions, we also measured many biochemical parameters as well as mechanical tests. Increased NO levels confirmed the effects of exogenous L-arginine and this fact was also verified by glutathione levels, since glutathione was also demonstrated to exert coronary vasodilation that was mediated by a nitric-oxide and guanylate cyclase dependent mechanism (10). Glutathione levels also gave an idea about the status of the oxidative stress within the groups (11).

MDA levels supported the hypothesis by demonstrating the lessened lipid peroxidation in L-arginine groups indicating tissue preservation as it was also confirmed by tha data of Yang et al. (12).

We aimed at answering many questions about exogenous L-arginine administration in this study based on an I/R design. We tried to understand the direct effects of L-arginine by measuring NO levels and verifying them by glutathione and MDA levels. The hemodynamic parameters mentioned in our study has also been investigated previously, but many of them were not confirmed by an appropriate biochemical data.

Consequently, we demonstrated that in a Langendorff model based on I/R, exogenous L-arginine administration improved mechanical and metabolic recovery of guinea pig hearts with respect to controls. We also suggest to use L-arginine before ischemia only and within a hypothermic solution, since we believe it has no additional beneficial effect when used in the repermsion period only and it has no cardioprotective effect in a normothermic setting.

We believe further studies, especially effects in the clinical setting, i.e. under extracorporeal circulation or within cardioplegic solutions should be designed to have more information on the clinical applications.

REFERENCES

1. Izhar U, Schwalb H, Borman JB, Merin G. Cardioprotective effect of L-arginine in myocardial ischemia and reperfusion in an isolated working heart model. J Cardiovasc Surg (Torino) 1998; 39(3): 321-9.
2. Braman RS, Hendrix SA. Nanogram nitrite and nitrate determination in enviromental and biological materials by vanadium (III) reduction with chemiluminescence detection. Analytical Chemistry 1989; 61:2715-18.
3. Kurtel H, Granger ON, Tso P, Grisham MB. Vulnerability of intestinal interstitial fluid the oxidant stress. Am J Physiol 1992; 263:573-8.
4. Casini A, Ferrali M, Pompella A, Maellaro E, Comporti M. Lipid peroxidation and cellular damage in extra-hepatic tissues of bromobenzene intoxicated mice. Am J Pathol 1986; 123:520-31.
5. Takeuchi K, Takashima K, Suzuki S, Fukui K. Basic amino acid, L-arginine aggravates ischemia-reperfusion injury. Nippon Kyobu Geka Gakkai Zasshi 1996; 44(2): 155-61.
6. Nonami Y. The role of nitric oxide in cardiac ischemia-reperfusion injury. Jpn Circ J 1997; 61(2): 119-32.
7. Engelman DT, Watanabe M, Maulik N, Engelman RM, Rousou JA, Flack JE, Deaton DW, Das DK. Critical timing of nitric oxide supplementation in cardioplegic arrest and reperfusion. Circulation 1996; 94(9):Suppl 11:407-11.
8. Amrani M, Gray CC, Smolenski RT, Goodwin AT, London A, Yacoub MH. The effect of L-arginine on myocardial recovery after cardioplegic arrest and ischemia under moderate and deep hypothermia. Circulation 1997; 96(9):Suppl 11:274-9.
9. Argano V, Galinanes M, Edmonson S, Hearse DJ. Effects of cardioplegia on vascular function and the no-reflow phenomenon after ischemia and reperfusiomstudies in the isolated blood reperfused rat heart. J Thorac Cardiovasc Surg 1996; 111(2):432-41.
10. Cheung PY, Schulz R. Glutathione causes coronary vasodilation via a nitric oxide and soluble guanylate cyclase dependent mechanism. Am J Physiol 1997; 273(3): H1231-38.
11. Massoudy P, Zahler S, Kupatt C, Reder E, Becker BF, Gerlach E. Cardioprotection by cyclosporin A in experimental ischemia and reperfusion-Evidence for a nitric oxide dependent mechanism mediated by endothelin. J Mol Cell Cardiol 1997, 29(2):535-44.
12. Yang BC, Mehta JL. Inhibition of nitric oxide does not affect reperfusion induced myocardial injury, but it prevents lipid peroxidation in the isolated rat heart. Life Sci 1997, 61(3):229-36.