

Effect of L-arginine on Function of Mitochondria in Ischemia – Reperfusion Myocardial Cell in Rabbits

Dan Chen¹, Junhui Zhou², Yingchun Ma¹, Haie Chen¹, Wantie Wang^{1*} and Lina Lin^{2*}

¹Department of Pathophysiology, Wenzhou Medical College, Wenzhou 325035, Zhejiang, China

²Department of Anesthesiology, First Affiliated Hospital of Wenzhou Medical College, Wenzhou 325003, Zhejiang, China

Abstract

Objective: To investigate the effect of the L-arginine (L-Arg) on function of the myocardial mitochondrial during myocardial ischemia - reperfusion (MIR).

Methods: Thirty rabbits were randomly divided into three groups (n =10 per group), namely control group, myocardial ischemia-reperfusion group (MIR) and L-Arginine pre-treated group (L-Arg +MIR). The relevant parameters, including myocardial mitochondrial respiratory function, Ca^{2+} concentration ($[Ca^{2+}]_m$), malondialdehyde (MDA) concentration, superoxide dismutase (SOD) activity, myocardial adenosine triphosphate (ATP), Adenosine diphosphate (ADP), adenosine monophosphate (AMP) content, the total amount of AMP (TAN), and energy charge (EC), were respectively determined.

Results: The mitochondrial respiratory control rate (RCR), III state respiration rate (V3), and SOD in L-Arg +MIR group were significantly higher than those of MIR group, while IV state respiration rate (V4), $[Ca^{2+}]_m$, and MDA were significantly lower than those of MIR group, myocardial ATP, ADP, TNA and the EC were significantly higher than those of group MIR; when compared with the group C, there was no significant difference in terms of V3, V4, SOD, MDA, and AMP, TAN between the L-Arg +MIR group and control group (group C).

Conclusion: It is indicated that L-arginine can reduce the level of the oxygen free radicals and attenuate calcium overload to improve the function of myocardial mitochondria during myocardial ischemia reperfusion injury.

Keywords: Ischemia; Reperfusion; The myocardium; Mitochondria; L-arginine

Abbreviations: L-Arg: L-arginine; MIR: Myocardial Ischemia-Reperfusion; MDA: Malondialdehyde; SOD: Superoxide Dismutase; ATP: Adenosine Triphosphate; ADP: Adenosine Diphosphate; AMP: Adenosine Monophosphate; TAN: The Total Amount of AMP; EC: Energy Charge; RCR: Respiratory Control Rate ; V3: III State Respiration Rate ; V4: IV State respiration rate

Introduction

Growing evidences from both animal experiments and clinical researches have shown that, L-arginine (L-Arg) has obvious effect on prevention and treatment for myocardial ischemia-reperfusion injury (MIR) [1-5]. To explore the effect of L-Arg on the myocardial mitochondrial function during MIR, the parameters as follows : mitochondrial respiratory function, Ca^{2+} concentration ($[Ca^{2+}]_m$), malondialdehyde (MDA) concentration, superoxide Dismutase (SOD) activity and adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) content, the total amount of AMP (TAN), energy charge (EC) intervention, were dynamically observed basing on the model, which provided a theoretical support for strengthening the perioperative cardiac protection.

Materials and Methods

Drugs and animals

L-arginine, purchased from Sigma Corporation..The 30 Japanese big-ear white rabbits, either male or female, weighting 2.0 ~ 3.0 kg, offered by Experimental Animal Center of Wenzhou Medical College. All animals received humane care in compliance with the European Convention on Animal Care.

Model duplication

Intravenous anesthesia was administrated with urethane (1.0g/kg), opened the chest and pericardium, ligated the left coronary artery with thread at the very place which was near left atrium about 5 mm to block the blood flow to form ischemia model, then cut the thread to reperfuse for 20 min after 40 min - ligation to form MIR model [1]. The alteration of ECG II was observed, the elevation of ST segment verified the existence of ischemia, and the deepening and widening Q wave meant the formation of reperfusion.

Experimental groups

The experimental rabbits were randomly divided into 3 groups (n = 10). In control group, the left ventricle coronary artery was separated without ligation and then the rabbits were sacrificed 1h later. In MIR group, the left ventricle coronary artery was ligated with thread to form complete cessation of blood flow for 40 min, and then 20 min-reperfusion was performed. In L-Arg+MIR group, the L-Arg (100mg/kg) was administrated earlier than 10 min before ligation for pre-

***Corresponding authors:** Lina Lin, Department of Anesthesiology, First Affiliated Hospital of Wenzhou Medical College, Wenzhou 325003, Zhejiang, China, Tel: +86-577-88069459; Fax: +86-577-88383956; E-mail: wzlinlina@tom.com

Wantie Wang, Department of Pathophysiology, Wenzhou Medical College, Wenzhou 325035, Zhejiang, China, Tel: 0577-86689817; Fax: 0577-86689817; E-mail: wzwwt@tom.com

Received April 06, 2012; Published June 22, 2012

Citation: Chen D, Zhou J, Ma Y, Chen H, Wang W, et al. (2012) Effect of L-arginine on Function of Mitochondria in Ischemia – Reperfusion Myocardial Cell in Rabbits. 1: 106. doi:[10.4172/scientificreports.106](http://dx.doi.org/10.4172/scientificreports.106)

Copyright: © 2012 Chen D, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

treatment via intravenous injection, and the period of reperfusion was same to MIR group. The blood sample and myocardial tissues were taken quickly after the reperfusion 20 min.

Isolated mitochondria

Mitochondria was isolated with sucrose gradient centrifugation. The components of the buffer as follows: sucrose (0.25 mol/L), Tris-hydrochloric acid buffer (10 mmol/L), pH 7.4 (prepared by deionized water). The operation was finished under the condition of 4°C and lasted 1h at least.

Mitochondrial respiratory function

Take sodium and sodium glutamate as the substrate, and apply BOM3 equipment to measure the oxygen consumption of myocardium for determining the function of mitochondrial respiratory and calculating the RCR and the respiratory rates of state III and IV (V3,V4) [6].

Statistical analysis

Data are presented as mean ± SE. Statistical analysis was performed using a one-way ANOVA. The exclusion of a normality (P > 0.05), the relation of data was analyzed by Pearson Correlation Procedure.

Results

L-arginine on mitochondrial respiratory function In MIR group, RCR, V3 were significantly lower than those of control group (P < 0.01), V4 significantly higher than that of control group (P < 0.01); RCR, V3 of L-Arg+MIR group were much higher than those of MIR group (P < 0.01 and P < 0.01), V4 obviously lower than that of MIR group (P < 0.05), and V3, V4 in control group showed no significant differences (Table 1).

L-arginine on mitochondrial Ca²⁺, MDA, SOD levels SOD activity in the MIR group was much lower than that of control group (P < 0.01), and the concentration of free Ca²⁺, MDA was much higher than that of control group (P < 0.01); the activity of SOD of L-Arg+MIR group was significantly higher than that of MIR group (P < 0.05), Ca²⁺, MDA levels were significantly lower than that of the group R (P < 0.05 and P < 0.01), and the activity of SOD, the content of MDA in control group showed no significant differences (Table 2).

L-arginine on myocardial EC, ATP, ADP, AMP and TAN levels ATP, ADP, TAN and the EC of MIR group were significantly lower than those of control group (P < 0.01), AMP was significantly higher than that of control group (P < 0.05); ATP, ADP, TAN and the EC of L-Arg+MIR group were significantly higher than those of group MIR group (P < 0.01), AMP of MIR group showed no significant difference (P > 0.05), while ATP, ADP, EC were still lower than those of control group (P < 0.05 and P < 0.01) (Table 3).

Myocardium of the relationship between the various indicators Linear correlation analysis showed that there was obviously negative correlation between the concentration of Ca²⁺ and RCR, EC of myocardial mitochondrial after 20-min reperfusion; the correlation coefficients were -0.631, -0.587, -0.612, 0.576, P < 0.01, “0.01,” 0.01, “0.01 respectively; there was also obviously negative correlation between the concentration of MDA in myocardial mitochondrial and RCR, EC, and the correlation coefficients were -0.865, -0.781, -0.655, 0.598, P < 0.01, “0.01,” 0.01, “ 0.01 respectively. There was obviously positive correlation between the activity of SOD in myocardial mitochondrial and RCR, EC, (coefficient = -0.521, -0.481, -0.426, 0.588, P < 0.01, “0.01,” 0.05, “ 0.01 respectively).

Discussion

The function of myocardial mitochondrial generating ATP efficiently by oxidative phosphorylation is crucial to myocardial metabolism. Among the parameters determining mitochondrial functions, the top two parameters known are the content of adenosine in myocardial and the synthetic ability of ATP in mitochondrial, followed by EC, RCR, and so on. This study finds that the abnormality of RCR, V3, V4, EC, ATP, ADP, AMP, TAN and abnormal changes of the function parameters of mitochondrial happened during cardiac-ischemia reperfusion. These changes mentioned above can be alleviated to different extent after L-Arg treatment, which indicates that there exist vary degrees of damage due to ischemia reperfusion on the respiratory function of myocardial mitochondrial; L-Arg can partly lessen the damage of mitochondrial during MIR, reduce disintegration of ATP and increase its synthetic ability to alleviate the failure of ATP in myocardial cells, and strengthen the energy reserve of myocardial cells [5,7], as well as recover the respiratory function of mitochondrial.

From Table 2, it showed that the levels of oxygen free radicals(OFR) in mitochondria during myocardial ischemia reperfusion increased and there existed calcium overload; after L-Arg treatment, the abnormality were alleviated to some extent; and there was obvious linear correlation between the concentration of the Ca²⁺ in mitochondrial, content of MDA, activity of SOD and the parameters reflecting the function of mitochondrial, which demonstrated that the damage of myocardial mitochondrial caused by ischemia reperfusion were related to calcium overload, oxygen free radicals (OFR) and the lipid peroxidation acted by OFR in mitochondrial, which was in line with the reports of Taylor and Paradies et al. [7,8].

L-Arg can reduce calcium overload, enhance the antioxidant capacity of mitochondrial and antagonize lipid peroxidation by taking part in the regulation of cardiomyocytes Ca²⁺ transportation [9-13]. L-Arg also improves the structure of mitochondria in myocardial cells in the reperfusion injury after myocardial ischemia by decreasing oxygen free radical level [14] and attenuate ischemia-reperfusion injury by antagonizing lipid peroxidation [8,9]. What's more, L-Arg can inhibit the expression of Fas/FasL mRNA, up-regulate bcl-2 mRNA and down-regulate bax mRNA expression in lung tissue [15], along with regulating the balance of bcl-2 mRNA and bax mRNA to decrease apoptosis [16], which plays notable protective role, thereby maintains the normal structure and function of mitochondrial. In addition, L-Arg can protect coronary endothelial cell and convert its dysfunction, it can raise the level of nitric oxide (NO) [2-3] in the

Group	RCR	V3 (nanoatom o•mg ⁻¹ •min ⁻¹)	V4 (nanoatom o•mg ⁻¹ •min ⁻¹)
Control	4.10 ± 0.17	148.78 ± 32.12	36.32 ± 7.98
MIR	1.94 ± 0.33**	101.14 ± 31.26**	51.23 ± 8.56**
MIR+ L-Arg	3.22 ± 0.43***	133.70 ± 32.67#	41.30 ± 7.84#

**P < 0.01 vs control group; #P < 0.05, ##P < 0.01 vs MIR group

Table 1: Effects of L-Arg on function of mitochondria in myocardial cell during MIR (x ± s, n = 10).

Group	Ca ²⁺ (nmol•mg ⁻¹)	MDA (nmol•mg ⁻¹)	SOD (U•mg ⁻¹)
Control	11.44 ± 2.40	1.23 ± 0.13	10.98 ± 2.89
MIR	23.43 ± 5.08**	1.75 ± 0.21**	7.04 ± 1.99*
MIR+ L-Arg	14.89 ± 4.41**#	1.33 ± 0.15##	9.70 ± 2.69#

*P < 0.05, **P < 0.01 vs control group; #P < 0.05, ##P < 0.01 vs MIR group

Table 2: Effects of L-Arg on Ca²⁺, MDA, SOD of mitochondria in myocardial cell during MIR (x ± s, n = 10).

Group	EC ($\mu\text{mol}\cdot\text{g}^{-1}$)	ATP ($\mu\text{mol}\cdot\text{g}^{-1}$)	ADP ($\mu\text{mol}\cdot\text{g}^{-1}$)	AMP ($\mu\text{mol}\cdot\text{g}^{-1}$)	TAN
Control	0.76 ± 0.02	6.77 ± 1.32	2.04 ± 0.53	1.45 ± 0.29	10.26 ± 2.05
MIR	0.58 ± 0.07**	2.86 ± 1.22**	1.12 ± 0.25**	1.84 ± 0.32*	5.83 ± 1.55**
MIR+ L-Arg	0.71 ± 0.02***	5.20 ± 1.29***	1.58 ± 0.41***	1.68 ± 0.29	8.47 ± 1.88**

* $P < 0.05$, ** $P < 0.01$ vs control group; # $P < 0.05$, ## $P < 0.01$ vs MIR group

Table 3: Effects of L-Arg on EC, ATP, ADP, AMP and TAN in myocardial tissue during MIR ($x \pm s$, $n = 10$).

body and reduce the level of endothelin to protect the mitochondria [2-3,10,17-19]. Besides, L-Arg is also an effective inhibitor for platelet adhesion and aggregation in the blood circulation during MIR [20], regulating the balance of thromboxane A2 and prostacyclin [21] and blocking the “no-reflow phenomenon”, which plays a significant role of mitochondria protection. Though growing studies confirmed that L-Arg works through the NO pathway, whether exogenous administration of NO is beneficial remains controversial. Of course, the obvious protective effect of L-Arg on mitochondrial will provide beneficial support for its clinical application.

Acknowledgement

This work was supported by Special fund of scientific research of young talent of health care in Zhejiang Province [Item number: 2001QN013].

References

1. Wantie W, Zhengjie X, Lina L (2005) Effect of ligustrazini on myocardial cell energy metabolism in the reperfusion injury after myocardial ischemia in rabbits. *Chin J Crit Care Med* 23: 129-130.
2. Wantie W, Lina L, Zhengjie X (2000) Role of nitric oxide in myocardial stunning after ischemia-reperfusion in rabbits and beneficial effect of L-Arginine. *Chin J Crit Care Med* 20: 576 -578.
3. Lei D, Daming G, Zhihao Y (2002) The protective effect of L-arginine on vascular endothelial cell during cardiopulmonary bypass. *Chin J Anesthesiol* 22: 35-38.
4. Lina L, Wantie W, Zhengjie X (2000) Protective effect of L-arginine on postischemia reperfusion myocardium during extracorporeal circulation. *Chin Clin J Thorac Cardiovasc Surg* 7: 89-91.
5. Lina L, Wantie W, Zhengjie X (2005) Effect of L-arginine on myocardial cell energy metabolism during the ischemia/reperfusion injury in rabbits. *Chin J Crit Care Med* 25: 417-419.
6. Poderoso JJ, Fernandez S, Carreras MC, Tchercanski D, Acevedo C, et al. (1994) Liver oxygen uptake dependence and mitochondrial function in septic rats. *Circ Shock* 44: 175-182.
7. Taylor PB, Lamb DR, Budd GC (1976) Structure and function of cardiac mitochondria in exhausted guinea pigs. *Eur J Appl Physiol Occup Physiol* 35: 111-118.
8. Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Serena D, et al. (1999) Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. *Free Radic Biol Med* 27: 42-50.
9. Wang WT, Wang W, Xu ZJ, Lin LN, Li D (2003) Role of lipid peroxidation in hepatic ischemia-reperfusion injury and effect of L-Arginine. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* 15: 91-93.
10. Cao J, Shi YR, Qi YF, Pang YZ, Tang CS (2002) Effects of mitochondrial L-arginine/nitric oxide system on mitochondrial Ca^{2+} transport in rat myocardium. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 18: 51-54.
11. Wantie W, Tao X, Zhengjie X, Keke J, Xuerong P, et al. (2004) Effect of L-arginine on function and structure of mitochondria in ischemia-reperfusion myocardial cells in rabbits. *Chin J Pathophysiol* 20: 2090-2094.
12. Wang WT, Hao ML, Wang FY, Ni SR, Song ZJ, et al. (2007) Effects of mitochondrial L-arginine/nitric oxide system on mitochondrial Ca^{2+} transport in rat myocardium. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 23: 134-137.
13. Wantie W, Fangyan W, Wei W, Tao X, Xuerong P, et al. (2006) Effect of ligustrazini and L-arginine on function of mitochondria in myocardial cell in the reperfusion injury after myocardial ischemia in rabbits. *Chin J Arterioscler* 14: 953-955.
14. Nonami Y, Rao V, Shiono N, Ogoshi S (1998) Quenching the effects of L-arginine on free radical injury in cultured cardiomyocytes. *Surg Today* 28: 379-384.
15. Wang WT, Wang XY, Chen RJ, Chen XW, Hao ML, et al. (2007) Effect of ligustrazini on expression of Fas/FasL in pulmonary injury induced by ischemia/reperfusion in rabbits. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 23: 87-91.
16. Wantie W, Yongyue D, Yixiao X, Xiaoxiao Q, Yang W, et al. (2007) Effect of L-arginine on expression of bcl-2 and bax mRNA in pulmonary injury induced by ischemia-reperfusion in rabbits. *Chin J Pathophysiol* 23: 288- 292.
17. Wantie W, Zhengjie X, Wei W (2004) Effect of L-arginine on nitric oxide and endothelin in the reperfusion injury after myocardial ischemia in rabbits. *Chin J Crit Care Med* 24: 423-424.
18. Xingsan L, Zhing Y, Zhiyu Z (2001) The protective effect of coronary vein retroperfusion of L-arginine on myocardial injury following ischemia in the pig. *J Cardiopulm Dis* 20: 109 -112.
19. Nakanishi K, Vinten-Johansen J, Lefer DJ, Zhao Z, Fowler WC 3rd, et al. (1992) Intracoronary L-arginine during reperfusion improves endothelial function and reduces infarct size. *Am J Physiol* 263: H1650-H1658.
20. Lina L, Wantie W, Xuerong P, Zhengjie X (2004) Effects of L-arginine on the function of platelet aggregation during hepatic ischemia/reperfusion injury. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* 16: 49-51.
21. Wang W, Xu Z, Lin L, Wang W, Li D, et al. (2000) Protective effect of L-arginine on liver during ischemia-reperfusion injury. *Zhonghua Gan Zang Bing Za Zhi* 8: 370-372.