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

Chen D1, Huang L1, Jinbo He1, Song D1, Yingchun Ma1, Lina Lin* and Wang W**
1Department of Pathophysiology, Wenzhou Medical University, Wenzhou 325035, Zhejiang, P.R.China
2Department of Anesthesiology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325003, and Zhejiang, P.R.China

Abstract
Objective: To investigate the effect of L-arginine (L-Arg) on the function of myocardial mitochondria during myocardia ischemia - reperfusion (IR).

Methods: Dividing randomly the 30 rabbits into three groups (n=10): control group (C), myocardia ischemia-reperfusion group (IR) and L-Arginine pretreatment group (L-Arg+IR). The relevant parameters, including myocardial mitochondria respiratory function, [Ca2+] concentration ([Ca2+]i), malondialdehyde (MDA) concentration, superoxide dismutase (SOD) activity, myocardial adenine triphosphate (ATP), Adenosine diphosphate (ADP), adenosine monophosphate (AMP) content, the total amount of AMP (TAN, TAN=ATP+ADP+AMP) and energy charge (EC, EC=1/2ADP+ATP/TAN) were determined, respectively.

Results: The mitochondria respiratory control rate (RCR), III state respiration rate (V3), and SOD in group L-Arg +IR were significantly higher than those in group IR, while IV state respiration rate (V4), [Ca2+]i, and MDA were obviously lower than those of group IR, the levels of ATP, ADP, TNA and the EC of myocardium showed significantly higher than those in group IR. There were no significant differences in terms of V3, V4, SOD, MDA, AMP and TAN between group L-Arg+IR and group C.

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

Keywords: L-arginine; Ischemia – reperfusion; Myocardium; Mitochondria

Abbreviations
L-Arg: L-Arginine; MIR: Myocardium 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 for myocardium ischemia-reperfusion (IR) injury [1-5]. To explore the effect of L-Arg on the function of myocardial mitochondria during IR, the parameters as follows: the respiratory function, Ca2+ concentration ([Ca2+]i), 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 of mitochondria were dynamically observed basing on the ischemia-reperfusion (IR) injury model, which provided a theoretical support for the cardiac protection in perioperative period.

Materials and Methods
Animals care, reagents and equipments
The study was approved by the Ethics Committee of Experimental Animals of Wenzhou Medical University of Zhejiang, China. The 30 Japanese big-ear male rabbits, weighing 2.0~3.0 kg, acquired from the Laboratory Animals Center of Wenzhou Medical University, all received humane care and all procedures fully complied with current ethical consideration. L-arginine (offered by Sigma Chemical Corporation, urethane (offered by Sigma Chemical Corporation), Tris-hydrochloric acid buffer 10 mmol/L, pH 7.4 (Invitrogen Co., USA), MDA and SOD kits (Jiancheng Bioengineering Research Institute, Nanjing, China), BOM3 equipment (Beijing No. 304 Hospital, China), High-pressure Liquid Chromatography (HPLC) (Beckman 322,USA).

Model duplication
The rabbits were randomly divided into 3 groups (n=10): control group I, IR group and L-Arg+IR group. For group IR, rabbits were anesthetized with urethane (1.0 g/kg body weight, i.v.), and opened the chest and pericardium, then the left ventricle coronary artery was ligated with thread to completely block the blood flow for 40 min, and followed by 20-min reperfusion through cutting the thread. The elevation of ST segment in ECG II meant myocardium ischemia, and the deepening and widening Q waves meant the reperfusion of myocardium [1]. In group C, the left ventricle coronary artery

*Corresponding author: Wanzhe Wang, Department of Pathophysiology, Wenzhou Medical University, Wenzhou 325025, Zhejiang, P.R. China, Tel: 0577-86689817; Fax: 0577-86689817; E-mail: wzwxt@tom.com
Lina Lin, Department of Anesthesiology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325003, Zhejiang, P.R.China, Tel: +86-577-88069459; Fax: +86-577-88383956; E-mail: wzlinlina@tom.com
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was separated without ligation, the rabbits were sacrificed at 60 min after the surgical procedure. For group L-Arg+IR, the rabbits were administrated with L-Arg (100 mg/kg, i.v.) 10 min prior to the ligation, the rest surgical procedure performed was the same to group IR. When myocardium reperfusion finished, all rabbits were sacrificed, the blood sample and myocardial tissues were immediately harvested.

**Isolated mitochondria**

Mitochondria were isolated with sucrose gradient centrifugation. The buffer was composed of sucrose (0.25 mol/L) and Tris-hydrochloric acid buffer (10 mmol/L), pH7.4 (prepared by deionized water). The performance was completed under the condition of 4°C within 1 hour.

**Mitochondria respiratory function**

Taking the sodium and sodium glutamate as the substrate, and applying the BOM3 equipment measured for oxygen consumption to calculate the respiratory control rate (RCR) and the respiratory rates of state III and IV (V3,V4) [6].

**Content of Ca\(^{2+}\) in mitochondria**

The mitochondria tissues were suspended in 1% HCl detected by ultrasonic extraction at 150W×10s for three times, centrifuged at 30000g for 60 min, then determined [Ca\(^{2+}\)] of the supernatant at 575 nm wavelength by OCP.

**MDA and SOD activity in mitochondria**

The content of MDA in mitochondria was detected by the thiobarbituric acid reactive substances with the results presented in unit of nmol/mg, the SOD activity was detected by the xanthine oxidase presented with unit of U/mg.

**Levels of ATP, ADP and AMP in myocardium**

The free wall and inferior-anterior wall of the left ventricle located under the ligation thread were homogenized in 5 ml HClO\(_4\) (0.6 mol/L), centrifuged at 1200 r/min×15 min at 4°C, then transferred and mixed the supernatant with K\(_2\)CO\(_3\) (1.2 mol/L). After a second centrifugation at 5000 r/min×10 min, supernatant was applied to determine the levels of ATP, ADP and AMP in myocardium by HPLC. (TAN=ATP+ADP+AMP, EC=1/2ADP+ATP/TAN).

**Statistical analysis**

All data were presented as mean ± SE. Statistical analysis was performed using a one-way analysis of variance (ANOVA). The relation of data was analyzed by Pearson Correlation Procedure. P<0.05 meant no significant difference.

**Results**

**The respiritory function of mitochondria**

In group IR, RCR and V3 were significantly lower than those of group C (P<0.01, P<0.01), V4 was significantly higher than that of group C (P<0.01). For group L-Arg+IR, RCR and V3 were much higher than those of group IR (P<0.01 and P<0.05), V4 obviously lower than that of group IR (P<0.05). V3 and V4 in group C and L-Arg+IR showed no significant differences (P>0.05, P>0.05) (Table 1).

**The levels of Ca\(^{2+}\), MDA and SOD in mitochondria**

Comparing with group C, the concentrations of Ca\(^{2+}\) and MDA in group IR were markedly higher (P<0.01, P<0.01), SOD activity was much lower (P<0.01). When compared with group IR, Ca\(^{2+}\) and MDA levels of group L-Arg+IR were significantly decreased (P<0.05, P<0.05), and SOD activities were significantly increased (P<0.05). The content of MDA and the activity of SOD between group C and group L-Arg+IR showed no significant differences (P>0.05, P>0.05), while the Ca\(^{2+}\) concentrations in group L-Arg+IR was higher than that of group C (P<0.01) (Table 2).

**EC, ATP, ADP, AMP and TAN levels of myocardium**

ATP, ADP, TAN and the EC of group IR were obviously lower than those of group C (P<0.01, P<0.01, P<0.01 and P<0.01). AMP was higher than that of group C (P<0.05). In group L-Arg+IR, the levels of ATP, ADP, TAN and the EC were higher than those of group IR (P<0.01, P<0.01, P<0.01, P<0.01), the level of AMP between group IR and L-Arg+IR showed no significant differences (P>0.05). When compared with group C, group L-Arg+IR showed lower levels of ATP, ADP, EC (P<0.05, P<0.05 and P<0.01) (Table 3).

**Linear correlation analysis between the indicators of Myocardium**

Linear correlation analysis indicated that the concentration of Ca\(^{2+}\) had negative correlation with RCR and EC of myocardial mitochondria after 20-min reperfusion (r=-0.631, -0.587, P<0.01, 0.01, respectively). The concentration of MDA showed obviously negative correlation with RCR and EC in myocardial mitochondria (r=-0.865, -0.781, P<0.01, 0.01, respectively). The activity of SOD showed positive correlation with RCR and EC in myocardial mitochondria (r=-0.521, -0.481, P<0.01, 0.01, respectively).

**Discussion**

The function of myocardial mitochondria on generating ATP efficiently by oxidative phosphorylation was crucial to myocardial metabolism. Among the parameters tested for mitochondria functions, the top two parameters known were the content of adenosine in myocardial and the synthetic ability of ATP in mitochondria, followed by EC, RCR. Our study found that the RCR, V3, V4, EC, ATP, ADP,
AMP, and TAN of mitochondria showed abnormal during cardiac-ischemia reperfusion. These changes mentioned above can be alleviated to some extent after L-Arg treatment, which indicated that the respiratory function of myocardial mitochondria were impaired due to IR. L-Arg can partly lessen the damage of mitochondria during IR, through reducing the disintegration of ATP and increasing its synthetic ability to alleviate the exhaustion of ATP in myocardial cells, and strengthen the energy reserve of myocardial cells [5-7], as well as recover the respiratory function of mitochondria.

From Table 2, the study showed that the levels of oxygen free radicals (OFR) in mitochondria increased during myocardial ischemia reperfusion, also calcium overloading occurred. After L-Arg treatment, the abnormality were alleviated to some extent, and there were obvious linear correlation between the concentration of the Ca\(^{2+}\), content of MDA, activity of SOD in mitochondria and the other parameters of mitochondria function, which demonstrated that the damage of myocardial mitochondria induced by ischemia reperfusion was related to calcium overload, oxygen free radicals (OFR) and the lipid peroxidation activated by OFR in mitochondrial. The findings were in line with the reports of Taylor and Paradie [7,8].

L-Arg can alleviate calcium overload, enhance the antioxidant capacity of mitochondria and antagonize lipid peroxidation by taking part in the regulation of cardiomyocytes Ca\(^{2+}\) transport [9-13]. L-Arg also improved the structure of mitochondria in myocardial cells during ischemia reperfusion by decreasing OFR level [14], and attenuated 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 tissues [15], as well as decrease apoptosis by regulating the balance between bcl-2 mRNA and bax mRNA [16], which played notable protective role of maintaining the normal structure and function of mitochondria. In addition, L-Arg can protect coronary endothelial cell and convert its dysfunction by raising the level of nitric oxide (NO) [2,3] and reducing the level of endothelin to avoid mitochondria injury [2,3,10,17-19]. Besides, L-Arg can also effectively inhibit platelet adhesion and aggregation during ischemia reperfusion [20], and regulate the balance of thromboxane A2 and prostacyclin [21] and block the no-reflow phenomenon, which played significant mitochondria protection. Though growing studies confirmed that L-Arg worked through the NO signal pathway, whether the exogenous administration on NO is beneficial remains controversial. Anyhow, the obvious protective effect of L-Arg on mitochondria may provide beneficial support for its clinical application.

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References


