Articles No. 1007-3949(2006)14-11-0953-03

•ORIGINAL ARTICLES•

Effect of Ligustrazini and L-arginine on Function of Mitochondria in Myocardial Cell in the Reperfusion Injury after Myocardial Ischemia in Rabbits

WANG Wan-Tie, WANG Fang-Yan, WANG Wei, XU Tao, PAN Xue-Rong, XIE Ke-jian, and FANG Zhou-Xi

(Department of Pathophysiology, Wenzhou Medical College, Zhejiang Wenzhou 325027, China)

[**KEY WORDS**] Ischemia-Reperfusion Injury; Myocardium; Mitochondria; Ligustrazini; L-arginine

Aim To study the effect of ligustrazini (LGT) and L-arginine (L-Arg) on function of mitochondria in myocardial cell during myocardial ischemia-reperfusion injury (MIRI).

Methods 50 animals were randomly divided into five groups (n= 10 in each): control group(A), MIR group (B), MIR+ LGT group (C), MIR+ L-Arg group (D), MIR+ LGT+ L-Arg group (E). The mitochondrial respiratory function, Ca^{2+} concentration([Ca^{2+}]m), malondialdehyde (MDA) content and superoxide dismutase (SOD) activity were determined. Meanwhile, the contents of ATP and EC in the myocardial tissue were measured, respectively.

Results It was found that mitochondrial respiratory control rate (RCR), state 3 (ST3), SOD, ATP and EC levels of myocardial tissue in C, D, E group were higher than those of B group, but state 4 (ST4), [Ca^{2+}]m, MDA were lower. However, between E and A group, all parameters had no significant differences.

Conclusion LGT and L-Arg can improve function of mitochondria in myocardial cells in the reperfusion injury after myocardial ischemia by decreasing oxygen free radical level and Ca^{2+} overload in the mitochondria.

1 INTRODUCTION

The effects of Ligustrazini (LGT), L-arginine (L-Arg) or coadministration of the two drugs on the mitochondrial respiratory function, Ca^{2+} concentration ([Ca^{2+}]m), malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, and the contents of ATP and energy charge (EC) in myocardial tissue were measured during myocardial ischemia-reperfusion injury (MIRI) in rabbits. The inner mechanism was investigated, which would provide a theory support for the protective functions of coadministration of LGT and L-Arg for hearts in preoperative period.

2 MATERIALS AND METHODS

2.1 Drugs and animals

Ligustrazine Hydrochloride Injection (Batch No 9610051, Drug Manufactory of Wuxi City Jiangchu Province), L-Arg (Sigma). 50 Japanese big ear white rabbits, either gender, weight 2.0~ 3.0 kg (the Experimental Animal Center of Wenzhou Medical College).

2.2 Animal model

Model of MIRI was built as described[1]. Intravenous anesthesia was administrated with urethane (1.0 g/kg). After chest and camera cordis was opened, the left coronary artery was ligated near auricula atrii sinistri about 5 mm to form ischemia. The thread was cut to reperfuse 20 min, after 40 min of ligation. ECG II changes was observed. The elevation of ST segment verified the exist of ischemia, and the deepeding and widening Q wave showed the formation of reperfusion.

2.3 Groups

Rabbits were randomly divided into five groups, ten rabbits each group. (1) Control group (A): operation was preformed but no ligation; (2) MIRI group (B); (3) MIRI+ LGT group (C): Ligustrazine Hydrochloride Injection (1.0 mL/kg) was given by i.v., prior to ischemia 20 min and just at the beginning of reperfusion, respectively. The rest steps were same to B group; (4) MIRI + L-Arg group (D): L-Arg (100mg/kg) was used by i.v., prior to ischemia 20 min. The rest steps were same to B group; (5) MIRI+ LGT+ L-Arg group (E): the two drugs was coadministratd by the same method and dose in C and D group, respectively. The rest steps were same to B group.

2.4 Preparation of mitochondria

Sucrose gradient centrifugation was used. Separating medium was prepaired by 250 mmol/L sucrose, 10 mmol/L tris-HCl buffer, pH 7.4 and deionized water. Under 4℃, abstraction of mitochondria was finished in one hour.

Project supported by: Foundation of Transcentury Academic and Technological Pioneer of Zhejiang Province and "551 Talented Man Projected" of Wenzhou City; Scientific Research Foundation of Zhejiang Provincial Health Department (No. 992086, 98113, 2001QN013)

Correspondence to Wan-tie WANG, Tel: 0577-86689817, E-mail: wzwwt@tom.com

2.5 Examination of mitochondrial respiratory function

Sodium malate and glutamic sodium as substrate, mitochondrial respiratory function was measured by BOM3 consumption of oxygen meter. Respiratory control ratio (RCR) and ⑶ ⑷state respiratory rate were calculated.

2.6 Determination of mitochondrial Ca^{2+} content

Elizabeth's method[3] slightly improved was utilized. Mitochondria were suspended in 1% HCl (volume ratio), and treated by 150 W supersound for 10 s three times, then centrifuged at 3 kr/min for 60 min. The supernatant was taken to colorate by orthocresol phenolphthalein complexone and shade selection was undertaken at 575 nm wavelength.

2.7 Determination of mitochondrial SOD and MDA

MDA content was examined by barbituric acid assay; SOD activity was determined by xanthine oxidase assay.

2.8 Determination of ATP and EC in myocardial tissue

At the end of reperfusion, myocardial tissue of free wall and inferior wall below the ligature was immediately harvested, and 5 mL $HClO_4$ (600 mmol/L) was added to make homogenate which was centrifuged at 12 kr/min for 15 min. The supernatant was taken back, and fully mixed with K_2CO_3 (1.2 mol/L) 1ml and centrifuged 5 kr/min for 10 min to retrieve the supernatant which was determined contents of ATP, ADP, AMP by high efficiency liquid chromatography (Beckman332 HPLC, USA). Consequently, the total quantity of adenine mononucleotide (TAN = ATP + ADP + AMP) was calculated. Energy state of cadiocyte was expressed by EC, the formula: EC = 1/2ADP + ATP/TAN.

2.9 Statistical Analysis

Data were reported as means ± SE, and analyzed using a one-way ANOVA. The relation of data was analyzed by Pearson process.

3 RESULTS

3.1 Mitochondrial respiratory function

RCR and ST3 were lower (B group vs A group, $P < 0.05$), higher (C, D, E group vs B group, $P < 0.01$ or $P < 0.05$), but no differences (C, D, E group vs A group $P > 0.05$). ST4 were much higher (B group vs A group, $P < 0.01$), significantly lower (C, D, E group vs B group, $P < 0.01$ or $P < 0.05$). But ST3 and ST4 were no differences (C, D group and, especially, E group vs A group $P > 0.05$) (Table 1).

3.2 Chages of mitochondrial Ca^{2+}, MDA, and SOD

SOD was much lower (B group vs A group, $P < 0.01$), higher (C, D, E group vs B group, $P < 0.05$ or $P < 0.01$). Ca^{2+} and MDA were lower (C, D, E group vs B group, $P < 0.05$ or $P < 0.01$). Meanwhile, there were no changes happened in the three indexes above in E group, compared with A group (Table2)

Table 1. The changes of myocardial mitochondrial respiratory function in different groups ($\bar{x} \pm s$, $n = 10$)

Groups	RCR	ST3[μmol/(g·min)]	ST4[μmol/(g·min)]
A	4.10 ± 0.17	148.8 ± 32.1	36.3 ± 8.0
B	1.94 ± 0.33[b]	101.1 ± 31.3[b]	51.2 ± 8.6[b]
C	3.24 ± 0.38[bd]	131.5 ± 28.9[c]	40.5 ± 7.6[d]
D	3.22 ± 0.43[bd]	133.7 ± 32.7[c]	41.3 ± 7.8[c]
E	3.76 ± 0.37[ad]	142.6 ± 32.9[d]	37.8 ± 6.5[d]

a: $P < 0.05$, b $P < 0.01$, vs A group; c$P < 0.05$, d$P < 0.01$, vs B group

Table 2. Changes of mitochondrial Ca^{2+}, MDA, and SOD in different groups ($\bar{x} \pm s$, $n = 10$)

Groups	Ca^{2+}(μmol/g)	MDA(μmol/g)	SOD(kU/g)
A	11.4 ± 2.4	1.23 ± 0.13	11.0 ± 2.9
B	23.4 ± 5.1[b]	1.75 ± 0.21[b]	7.0 ± 2.0[b]
C	14.9 ± 4.4[ad]	1.36 ± 0.16[ac]	9.4 ± 2.6[c]
D	14.9 ± 4.4[bc]	1.33 ± 0.15[d]	9.7 ± 2.7[c]
E	12.9 ± 2.8[d]	1.26 ± 0.15[d]	10.5 ± 3.0[d]

a: $P < 0.05$, b: $P < 0.01$, vs A group; c: $P < 0.05$, d: $P < 0.01$, vs B group

3.3 Changes of EC and ATP in myocardial tissue

ATP and EC decreased (B group vs A group, $P < 0.01$), increased (C, D, E group vs B group, $P < 0.01$). Furthermore, the two indexes of E group were no statistical differences in contrast of A group ($P > 0.05$) (Table 3).

Table 3. Changes of EC and ATP in myocardial tissue in different groups ($\bar{x} \pm s$, $n = 10$)

Groups	EC	ATP (μmol/g)
A	0.76 ± 0.02	6.77 ± 1.32
B	0.58 ± 0.07[b]	2.86 ± 1.22[b]
C	0.70 ± 0.03[bd]	5.05 ± 1.34[ad]
D	0.71 ± 0.02[bd]	5.20 ± 1.29[ad]
E	0.74 ± 0.03[d]	6.25 ± 1.46[d]

a: $P < 0.05$, b: $P < 0.01$, vs A group; c: $P < 0.05$, d: $P < 0.01$, vs B group

3.4 Relation of the data in myocardial tissue

Linear correlation analysis showed that after 20 min reperfusion, there existed obvious negative correlativity between mitochondrial Ca^{2+} content and RCR, EC ($r = -0.631$, -0.587; $P < 0.01$, respectively), between mitochondrial MDA content and RCR, EC ($r = -0.865$, -0.781; $P < 0.01$, respectively); and mitochondrial SOD activity positively correlated with RCR and EC ($r = -0.521$, -0.481; $P < 0.01$, respectively).

4 DISCUSSION

The main function of myocardial mitochondria is efficiently to convert metabolin in kytoplasm into ATP by oxidative phosphorylation. Among the parameters measuring mitochondrial functions, adenine nucleotide content in myocardial tissue and the synthesis ATP capability of mitochondria, which both are often reflected by RCR and EC, respectively, are the most important. In this study, the obvious abnormality of RCR, ST3, ST4, EC and ATP etc was caused by MIRI, but improved in different extents, after treatment of LGT AND L-Arg. This indicated that mitochondrial respiratory function of cadiocytes was damaged, during ischemia-reperfusion; while LGT and L-Arg which can partly lessen damage of mitochondria reduced degradation, increased production and delay exhaustion of myocardial ATP so that energy store was strengthened and mitochondrial respiratory function was improved[6,7].

From Table 2, we knew that LGT and L-Arg can weaken the increase in oxygen free radical and overload of Ca^{2+} caused by MIRI. Furthermore, mitochondrial Ca^{2+} concentration, MDA content and SOD activity were well related with each other. So we can conclude that damage of mitochondria may concern with Ca^{2+} overload, oxygen free radical and lipid peroxidation reaction. The conclusion is same to Paradies' s report[8]. LGT and L-Arg can adjust the mitochondrial transport of Ca^{2+} to lessen Ca^{2+} overload, improve antioxadation capacity of mitochondria to decrease the lipid peroxidation damage[9,10] so as to maintain normal structure and function of mitochondria. Certainly, LGT and L-Arg coadministration can effectively suppress adhere and aggregation of platelet, adapt the balance of TXA2/PGI2[11-13], and lessen "no reflowing phenomenon" to protect mitochondria.

[Reference]

[1] Wang WT, Lin LN, Xu ZJ, et al. Role of nitricoxide in myocardialstunning after ischemia-reperfusion in rabbit and benidicial effect of L-Arginine. *Chin J Crit Care Med*, 2000, **20:** 576-578

[2] Poderoso JJ, Fernadez S, Carreras MC, et al. Liver oxygen uptake dependence and mitochondrial function in septic rats. *Circ Shock*, 1994, **44:** 175-179

[3] Elizabeth AF, James DM, takuro T, et al. Myocardial mitochondrial calcium accumulation modulates nuclear calcium accumulation and DNA fragmentation. *Ann Thorac Surg*, 1995, **60:** 338-344

[4] McCallister LP, Page E. Effects of thyroxin on ultrastructure of rat myocardial cells: astereological study. *J Ultrastr Res*, 1973, **42:** 136-141.

[5] Smith HE, Page E. Morphometry of rat heart mitochondrial subcompartments and membranes. *J Ultrastr Res*, 1976, **55:** 31-37.

[6] WANG WT, XU ZJ, LIN LN, et al. Effect of ligustrazini on myocardial cell energy metabolism in the reperfusion injury after myocardial ischemia in rabbits. *Chin J Crit Care Med*, 2003, **23:** 129-130

[7] Lin LN, Wang WT, Xu ZJ. Effect of L - arginine on myocardial cell energy metabolism during the ischemia/ reperfusion injury in rabbits. *Chin J Crit Care Med*, 2005, **25:** 417-419

[8] Paradies G, Petrosillo G, Pistolese M, et al. Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. *Free Radic Biol Med*, 1999, **27:** 42-50

[9] Xu ZJ, Wang WT, Li D. Protective Effect s of Liqustrazin on Myocardial Ischemia-Reperfusion Injury in Rabbits and Its Mechanism. *Basic Med Scien Clin*, 1997, **17:** 702-704

[10] Wang WT, Wang W, Xu ZJ, et al. Role of lipid peroxidation in hepatic ischemia-reperfusion injury and effect of L-Arginine. *Chin Crit Care Med*, 2003, **15:** 91-93

[11] Wang WT, Lin LN, Xu ZJ. The effect of Liqustrazin on platelet aggregation function of patients changing heart valvula. *CJITWM*, 2000, **20:** 25-26

[12] Xu ZJ, Wang WT, Xie KJ. The balance changes of PGI2/TXA2 during Myocardial Ischemia-Reperfusion Injury and Liqustrazin' s regulatory function on it. *J Wenzhou med col*, 1997, **27:** 129-131

[13] Wang WT, Xu ZJ, Lin LN, et al. protective effect of L-Arginine on liver during ischemia-reperfusion injury. *Chi J Hepatol*, 2000, **8:** 370-272

川芎嗪联合左旋精氨酸对缺血-再灌注损伤兔心肌线粒体功能的影响

王万铁[1], 王方岩[1], 王 卫[1], 许 涛[4], 潘雪蓉[1], 谢克俭[2], 方周奚[3]

(温州医学院 1. 病理生理学教研室, 2. 检验分析中心, 3. 电子显微镜室, 浙江省温州市 325027;
4. 安徽中医学院病理学教研室, 安徽省合肥市 230038)

[关键词] 病理学与病理生理学; 缺血-再灌注损伤; 心肌; 线粒体; 川芎嗪; 左旋精氨酸

[摘 要] **目的** 探讨川芎嗪联用左旋精氨酸对心肌缺血-再灌注损伤时心肌细胞线粒体功能的影响。**方法** 选用日本大耳白兔 50 只, 随机分为正常对照组(A 组)、心肌缺血-再灌注组(B 组)、心肌缺血-再灌注+川芎嗪治疗组(C 组)、心肌缺血-再灌注+左旋精氨酸治疗组(D 组)和心肌缺血-再灌注+川芎嗪+左旋精氨酸治疗组(E 组)。观察心肌线粒体呼吸功能、Ca^{2+} 浓度、丙二醛浓度、超氧化物歧化酶活性和心肌组织三磷酸腺苷(ATP)和能荷的变化。**结果** 与 A 组比较, B 组线粒体呼吸控制率、Ⅳ态呼吸速率和超氧化物歧化酶明显降低, Ⅴ态呼吸速率、Ca^{2+} 浓度和丙二醛显著升高, 心肌组织 ATP 和能荷明显降低。与 B 组比较, C 组、D 组和 E 组线粒体呼吸控制率、Ⅳ态呼吸速率和超氧化物歧化酶明显升高, Ⅴ态呼吸速率、Ca^{2+} 浓度、丙二醛显著降低, 心肌组织 ATP 和能荷明显增高; 且与 A 组比较, E 组上述指标均无明显差异。**结论** 川芎嗪联用左旋精氨酸可通过降低氧自由基水平和减轻钙超载, 而改善缺血-再灌注损伤心肌的线粒体功能。

(此文编辑 胡必利)