脂质胞壁酸诱导的延迟预适应对大鼠局灶性脑缺血 /再灌注损伤的保护作用

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摘要:目的 探讨 LTA诱导的延迟预适应对大鼠局灶性脑缺血 /再灌注损(I/R)损伤的作用。方法 采用改良 Longa法制作大鼠右大脑中动脉(MCA)闭塞 2 h造成局灶性脑缺血模型,恢复血液灌流 24 h。大鼠在施行脑缺血前 24 h腹腔注射脂质胞壁酸(LTA,1 mg/kg)诱导延迟预适应,检测脑组织再灌注 24 h后组织中超氧化物歧化酶(SOD)、丙二醛(MDA)和一氧化氮(NO)含量以及大鼠神经症状,并用透射电镜观测大鼠大脑皮层神经细胞的超微结构改变。结果 LTA预适应能明显减少脑 I/R后组织中 MDA的含量($P<0.01$),提高 SOD的水平($P<0.01$),减轻神经细胞的超微结构损伤和保护细胞膜结构完整性,LTA预适应还能明显改善脑 I/R后的神经功能,减少神经缺欠评分值($P<0.01$)。LTA预适应亦能明显降低 I/R导致脑组织中 NO含量的升高($P<0.01$)。结论 LTA诱导的延迟预适应能显著减少大鼠脑组织再灌注损伤,减少脑组织坏死,其作用机制与减少脑 I/R后自由基和 NO毒性作用有关。

关键词:脑缺血;再灌注损伤;脂质胞壁酸;预适应;一氧化氮
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INTRODUCTION

Clinically, in cerebral aneurysm surgical procedures, temporary brain artery occlusion is often used to facilitate surgical access and reduce bleeding. Temporary vessel occlusion following reperfusion might produce focal cerebral reperfusion injury. If neurosurgical patients with possible temporary vessel clipping are preconditioned with treatment, cerebral damage might be prevented. On the other hand, although experimental studies have been

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suggested that " therapeutic window" for reflow blood in focal cerebral ischemia can as long as 6-8 h in the primate and 2-3 h in the rat. Li et al reported that there were some intact neurons in the ischemic core 46 h after 2 h of MCA occlusion[1]. These findings have suggested that delayed therapeutic interventions might be an additional effect for protection cerebral injury.

Preconditioning (PC) has been confirmed as a universal mechanism to protect tissues from an impending threat. Ischemic PC-induced tolerance against ischemic injury in the brain was first reported by Kitagawa et al in 1991. This remarkable phenomenon can lasts for several hours and appears after 1-7 days. Such delayed neuroprotection is also induced by heat stress, isoflurane, lipopolysaccharide (LPS)[2,3]. Lipoteichoic acid (LTA) is a cell wall component of Gram-positive organisms, containing a substituted polyglycerophosphate backbone attached to a glycolipid, equivalent to LPS in Gram-negative bacteria, but could not initiate Gram-negative septic shock. Zacharowski and colleagues for the first time demonstrated that pretreatment of rats in vivo with LTA (1 mg/kg, ip) for 8 to 24 h significantly reduces infarct size and cardiac troponin T (cTnT) release in rats subjected to myocardial left anterior descending coronary artery (LAD) occlusion-reperfusion[4]. However, there is no report to study that LTA pretreatment can protect cerebral reperfusion injury. The aim of this study was first to explore the effects of LTA induced delayed PC against cerebral reperfusion injury following middle cerebral artery (MCA) occlusion in rats.

1 MATERIAL AND METHODS

1.1 Model of cerebral ischemia-reperfusion

Male Wistar rats (Grade, certificate No. 19-050, obtained from Department of Experimental Animal, Tongji Medical College, weighting 280 to 330g) were provided free access to food and water before and after surgery. Rat were anesthetized with chloral hydrate (350 mg/kg, ip) and allowed to breathe spontaneously. Focal cerebral ischemia was made by means of right MCA occlusion by the modified method of Longa et al[5]. Briefly, after median incision of the neck skin, the right common carotid artery (CCA), internal carotid artery (ICA), external carotid artery (EAC) were carefully exposed and isolated. The distal portion of the ECA was ligated using 4-0 silk sutures. The CCA dissected just below the carotid bifurcation and a 4-0 nylon thread (with heat-blunted tip by heating near a flame) was inserted into the ICA approximately 17 18 mm distal to the carotid bifurcation until a slight resistance was felt, thereby occluding the origins of the anterior cerebral artery, the MCA, and the posterior communicating artery. Then, the skin was closed with 3-0 silk running sutures. After 2 h of MCA occlusion, the thread was carefully removed until its tip was blocked by ligature placed on common carotid artery to permit reperfusion. Rats that exhibited convulsions, that sustained consciousness disturbances, or that were without neurologic deficits 30 min after recovery from anesthesia were excluded from the study.

1.2 Experimental protocols

Rats were randomly allocated into the following groups: (1) Sham group: Rats were pretreated with saline (5mL/kg ip) 24 h before the experiment, and then subjected to sham operation. (2) Ischemia/reperfusion (I/R) group: Rats were pretreated with saline 24 h before the experiment, and then performed MCA occluded for 2 h following 24 h of reperfusion. (3) LTA group: Rats were administrated with LTA (1 mg/kg, ip) 24 h before the experiment, and then subjected to ischemia and reperfusion. After experience, the rats were killed by decapitation, and the brains were quickly removed to collect the cerebral tissues. The ischemic right brain tissues were separated and frozen immediately in liquid nitrogen and stored at -70℃ until further processing.

1.3 Neurological evaluation

Neurologic deficit examination was performed on each rat at 24 h after reperfusion by held the rat tail according to the scoring system on the basis of the severity of the following symptoms: truncal curvature, circulating behavior and rolling fit, all considered being typical for stroke. A standard scoring scale was used[6]: 0, no deficit; 1, failure to extend left forepaw fully; 2, circling to the left; 3, falling to the left; 4, no spontaneous walking, with a depressed level of consciousness; 5, dead.

1.4 Measurement of the levels of superoxide dismutase (SOD) and malondialdehyde (MDA) in cerebral tissues

For reflection the state of lipid peroxidation of membrane in cerebral tissues, the extents of MDA and SOD in right cerebral tissues at the end of reperfusion were determined by chromometry using MDA and SOD assay kits which purchased from Jiancheng Bioengineering Institute (Nanjing, China). SOD was measured as enzyme activity (U/mL). The protein content was determined by Coomassie brilliant blue G-250 staining assay using BSA as standard.

1.5 Measurement of nitric oxide products in cerebral tissues

The production of NO was determined indirectly by measuring NO_2^- and NO_3^- in right cerebral tissue after different treatment. The products were measured by NO assay kit according to the manufacturer's instructions.

1.6 Electron microscopic observation

A rat for each group was anesthetized at the end of reperfusion for 24 h and were perfused with 2% glutaraldehyde and 2% paraformaldehyde (in 0.15 mol/L sodium phosphate-buffered saline, pH 7.4) through the ascending aorta. The right cerebral cortex were removed and placed in the same fixative for at least 12 h. Coronal ultrathin sections stained with 2% uranyl acetate and 1% lead citrate and examined under an H-600 electron mi-

croscope.

1.7 Statistics

Data were expressed as mean SD. Comparisons between the 2 experimental groups were made by Student's t test. Neurologic deficit scores were analyzed with the U-test. Statistical significance was accepted at the 95% significance level ($P < 0.05$).

2 RESULTS

2.1 Neurological evaluation, the contents of SOD and MDA and the level of NO products in cerebral tissues after MCA occlusion following reperfusion or LTA pretreatment

All rats in I/R group showed moderate or severe neurological deficit after 24 h reperfusion with a mean score of 2.29 1.03. Neurological function was significantly improved in the LTA-treated rats with a mean score of 0.71 0.70 ($P < 0.01$) (Tab 1). The concentration of MDA and the level of NO at the end of reperfusion in I/R group was much higher than in sham group ($P < 0.01$), and the activity of SOD was lower than in sham group ($P < 0.01$). Compared with in I/R group, LTA pretreatment was obviously decreased the content of MDA and the concentration of NO, and increase the level of SOD ($P < 0.01$). The concentrations of MDA and SOD have no significant difference between in LTA group and in sham group (Tab 1).

2.2 The Change of ultrastructural in neuron of right cerebral cortex after MCA occlusion following reperfusion or LTA pretreatment

In sham group, the cellular structure of neuron was integrity, and the mitochondria and Golgi apparatus were clearly visible. The nucleus was ovoid in shape and the nucleolus was prominent and the chromatin granules were scattered in the nucleus (Fig 1A). In comparison with the sham group, the ultrastructure of the neuron exhibited dramatic changes after reperfusion. Many vacuoles and a dilated mitochondrion (arrow) could be found in the cytoplasm. The small and irregular chromatin aggregates were scattered in the nucleus and the nuclear membrane was fragmented and dissolved (Fig 1B). While in LTA pretreatment rat, the cellular structure of neuron was nearly integrity, and the mitochondria and Golgi apparatus were visible although some mitochondria became flocculent dense bodies and had little swollen. The nucleolus was prominent and the chromatin granules were scattered in the nucleus (Fig 1C).

Tab 1 Effects of lipoteichoic acid (LTA) induced delayed preconditioning on neurologic deficit scores, the contents of malondialdehyde (MDA), superoxide dismutase (SOD) and nitric oxide (NO) in cerebral tissues after the right middle cerebral artery (MCA) occlusion for 2 h following reperfusion for 24 h in rat. $n = 7$, mean SD. LTA: (1 mg/kg, ip).

表 1 LTA诱导的延迟预适应对大鼠缺血 2h再灌注 24h后对神经症状缺乏 ,组织 MDA和 SOD的影响及血浆一氧化氮 (NO)的作用 ($\bar{x} \pm s$, $n = 8$)

Group	Neurologic Score	MDA [μmol/g(pro)]	SOD [$\times 10^3$, U/g(pro)]	NO [μmol/g(pro)]
Sham	0	0.90 ±0.16	3.54 ±0.31	0.396 ±0.045
I/R	2.29 ±1.03	1.83 ±0.33*	2.72 ±0.38*	0.892 ±0.091*
LTA	0.71 ±0.70**	1.27 ±0.21**	3.68 ±0.40**	0.621 ±0.069**

Note: * $P < 0.01$ vs sham group, ** $P < 0.01$ vs I/R group

注 :与假手术组比较 , * $P < 0.01$;与缺血再灌注组比较 , ** $P < 0.01$

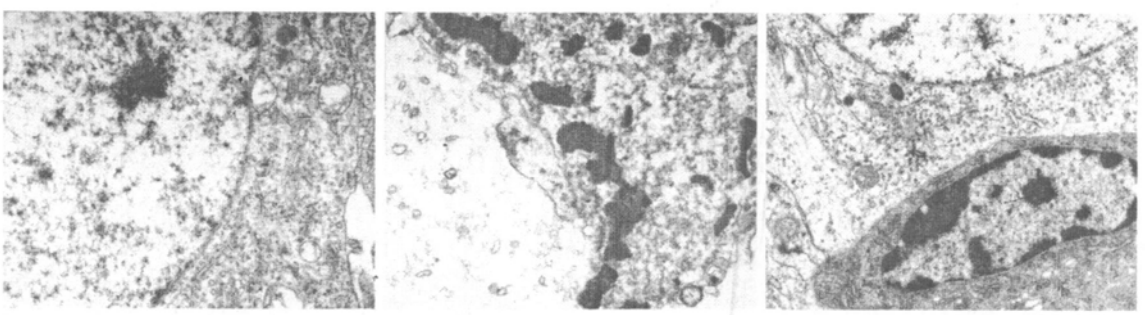

Fig 1A Fig 1B Fig 1C

Fig 1 In sham group, the cellular structure of neuron is integrity, and the mitochondria and Golgi apparatus are clearly visible. The nucleus is ovoid in shape and the nucleolus is prominent and the chromatin granules are scattered in the nucleus (Fig 1A. ×15 000). In I/R group, many vacuoles and a dilated mitochondrion (arrow) could be found in the cytoplasm. The small and irregular chromatin aggregates are scattered in the nucleus and the nuclear membrane is fragmented and dissolved (Fig 1B. ×15 000). While in LTA pretreatment rat, the cellular structure of neuron is nearly integrity, and the mitochondria and Golgi apparatus are visible although some mitochondria became flocculent dense bodies and have little swollen. The nucleolus is prominent and the chromatin granules are scattered in the nucleus (Fig 1C. ×15 000).

图 1 在假结扎组 ,神经细胞结构完整 ,线粒体 、高尔基体清晰可见 ,细胞核呈卵状 ,核仁明显 ,染色质颗粒分散于细胞核 (图 1A. ×15 000)。在结扎再灌注组 ,细胞质中可见液泡和膨大的线粒体 ,染色质呈小而不规则聚集体分散于细胞核 ,核膜破碎 、溶解 (图 1B. ×15 000)。在 LTA处理组 ,神经细胞结构基本完整 ,尽管有部分线粒体呈絮凝密集体并稍显肿胀 ,但线粒体 、高尔基体仍清晰可见 ,核仁明显 ,染色质颗粒分散于细胞核 (图 1C. ×15 000)。

3 DISCUSSIONS

The present study showed that the content of MDA was raised and the activity of SOD was decreased. At the same time, I/R could result neurological deficit symptom in rat. As we know, excessive oxygen free radical (OFR) induced by I/R was shown to be a final pathway in ongoing cell death. However, LTA pretreatment could obviously decrease the production of MDA and increase the activity of SOD and improve the neurological symptom. Although we did not assess infarct size in the present study, it also suggested that the delayed PC induced by LTA reduced necrosis that caused by peroxidate reaction, and neuroprotection through anti-free radical effect.

Although it is not clearly demonstration the role of NO in the pathophysiological mechanisms in cerebral I/R and PC, much evidence suggested that endothelial NOS produces NO with beneficial effects, such as vasodilatation, reduction of endothelial inflammation, and direct quench free radicals generated during ischemia and reperfusion in brain, whereas nNOS and iNOS appear to be deleterious[7]. These deleterious effects may associate with its reaction with superoxide anions ($O_2^{\cdot}$) to generate peroxinitrite anion ($ONOO^-$) that can theoretically rapidly decompose to form a hydroxyl radical ($\cdot OH$) or some other potent oxidant and contribute to oxidize proteins, lipids and DNA. The present study showed that cerebral I/R could obviously increase NO production and accompany a raise in the content of MDA and number of apoptotic cells. It confirms the opinion described above.

However, in our previous study, LTA induced delayed PC could increase NO production and obviously protect myocardium against I/R injury in isolated rat heart, and inhibition of endogenous of NOS by L-NAME (non-selective NOS inhibitors) pretreatment could abolish the protection induced by LTA pretreatment[8]. The delayed PC induced by LTA could significantly decrease the overproduction of NO and protect the cerebral reperfusion injury, it suggested that decrease NO is one of mechanism of LTA preconditioning. Since I/R induced oxidative stress could stimulate the nNOS and iNOS induced a high production of NO, LTA pretreatment could obviously decrease the production of OFR and increase the activity of SOD, thus it suggested LTA preconditioning could reduce the extent of oxidative stress, and decrease the iNOS and nNOS-derived NO as a result. It also suggested that some aspects of the mechanism of LTA induced PC are not universal for all tissue types and NO played a dual role in the pathophysiology of LTA preconditioning in the different models.

In conclusion, the present study provided evidence for the first time that LTA induced delayed PC could reduce cerebral I/R injury. The neuropective mechanisms of LTA pretreatment may be associated with anti-free radical effect and decreased the cytotoxic effect of NO.

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