Preventive Effect of Fastigial Nucleus on Oxidative Damage in Rats Undergoing Acute Myocardial Infarction

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The fastigial nucleus (FN) plays an important role in regulating cardiovascular, respiratory, and visceral activities. We investigated the effect and mechanism of FN stimulation (FNS) on the prevention of oxidative damage in wistar rats undergoing surgically induced acute myocardial infarction (AMI). The FN lesion (FNL) group was subjected to bilateral FN lesioning before the nucleus was electrically stimulated and AMI was induced. FN of AMI rats were electrically stimulated as the FNS and AMI group. Sham-stimulated and sham-operated rats were randomly selected in one group as control. We measured lactic dehydrogenase (LDH) and creatine phosphokinase (CK) activity, as well as myocardial infarction (MI) size. Malondialdehyde (MDA) content, total anti-oxidative capability (TAOC) and superoxide dismutese (SOD) activity in myocardium were also measured. FNS lowered the activities of LDH and CK in comparison to the AMI group. Infarct size was less in FNS-treated rats. The AMI group had elevated MDA levels compared with sham-treated animals. TAOC and SOD activities were decreased in the AMI group; there was attenuation of MDA and increases of TAOC and SOD activities in the FNS group ($p < 0.01$). We conclude that FNS can reduce damage during MI. The mechanism of the protective effect might be partially related to its antioxidative role.

Key words —— fastigial nucleus, electrical stimulation, myocardial infarction, oxidative damage

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INTRODUCTION

Some studies have shown that fastigial nucleus (FN) plays an important role in regulating cardiovascular, respiratory, and visceral activities.[1] FN stimulation (FNS) can protect vital organs such as the brain and heart from ischemic injury.[2] It was recently reported that electrical stimulation of the FN decreased the volume of cerebral infarction, inhibited leukocyte infiltration into ischemic tissue, suppressed the inflammatory response in cerebral microvessels, and reduced production of oxygen-derived free radicals (OFR) as well as neuronal death after ischemia.[3] However, it was not clear whether electrical stimulation of the FN has protective effects against myocardial infarction (MI). The goal of this study was to explore the potential therapeutic advantage of FNS for MI by observing oxidative damage in the myocardium after electrical stimulation of the FN in rats with MI.

MATERIALS AND METHODS

Animals and Reagents —— A total of 40 healthy male Wistar rats (9–11 weeks old, weighing 250 to 300 g) were used in the study. All the rats used in the following experiments were subject to the Guiding Principles for the Care and Use of Laboratory Animals and the Recommendations from the Declaration of Tongji University. The rats were randomly divided into four groups. Acute myocardial infarction (AMI) was induced in the AMI group ($n = 9$). The second group (FNS) received FNS for 1 hr before induction of AMI ($n = 11$). The third group FN lesion (FNL) of rats underwent lesioning of the FN five days before receiving FNS and AMI ($n = 10$). Sham-operated rats ($n = 10$) were randomly selected as a non-infarction control group. These rats did not receive electrical stimulation, lesioning, or ligation. Kits were used to measure malondialdehyde (MDA), total anti-oxidative capability (TAOC), superoxide dismutase (SOD), lactic dehydrogenase (LDH), and creatine phosphokinase (CK). These reagent kits were purchased from the Institute of Bioengineering (Nanjing Jiancheng, China).

Electrical Stimulation of FN —— Electrical stimulation of FN was performed using the methods described by Reis *et al.*[4] Each rat was anesthetized and placed in a stereotaxic instrument. A median incision was cut at top of the head to expose the skull. The FN was identified using an atlas of the Wistar

rat brain[5] and the electrode was lowered into the FN. The FN was then electrically stimulated (70 Hz frequency) for 1 hr.

Chemical Lesions of FN —— Bilateral FN neurons were destroyed by injecting 0.2 μl ibotenic acid with 0.5 μl Hamilton microsyringe. After the tubes were loaded with 50 mM ibotenic acid, the injection was completed over 30 s. The procedure was repeated on the contralateral side. Only one volume was injected each time as described by Glickstein *et al.*[6] The effectiveness of chemical lesion was verified by observing Nissl stained brain slices and the dramatic effects of this neurotoxin on posture and locomotion of rats after the lesion was created.

Induction of MI —— MI was created by ligating the left anterior descending artery (LAD). In brief, the thoracic cavity was opened under ventilator-improved respiration, the heart was exteriorized, and the pericardium was incised. Thereafter, the LAD was ligated 2 mm from its origin between the left auricle and the pulmonary outflow tract with 6–0 silk threads. The thoracic cavity was then closed. The effectiveness of the MI was demonstrated using an electrocardiogram (ECG), in which ST section in leads I, avL had raised for 30 min. Blood samples and a sample of the left ventricular myocardium were collected 24 hr after MI. At the conclusion of the experiment, MI was confirmed by histological examination using nitroblue teltrazolium (NBT) staining.

Measurement of Infarct Size —— After removal of the right ventricle and atria, the hearts were snapping frozen in liquid nitrogen until analyzed. The left ventricle was cut into serial sections (1 cm thick) perpendicular to the apex-base axis. The slices were then incubated at 37°C for 20 min in 5% nitroblue tetrazolium (pH 7.4). NBT stains the non-infarcted myocardium blue, indicating the presence of a formazin precipitate that results from the reduction of NBT by dehydrogenase enzymes in viable tissue. The infarcted region was not stained since it lacks the dehydrogenase enzyme. The non-stained and the blue-stained regions were carefully separated using a dissecting microscope. The total ventricular and the infarcted tissue weights were determined by electronic balance. The infarct size was calculated as a percentage of the total left ventricular weight.

Determination of Myocardial MDA, TAOC, and SOD —— A 0.5 g piece of the myocardium was homogenized in 4.5 ml of ice-cold physiological saline. The homogenates were centrifuged and the pellet used for measuring tissue MDA content, SOD activity, and TAOC using a chemical colorimetric method that was performed according to the manufacturer instructions.

Determination of Serum LDH and CK —— In LDH and CK activity of the rat, marked interlaboratory variation exists in the analysis. To limit variation and avoid inappropriate sampling and handling techniques, we used the method described previously by Matsuzawa *et al.*[7] In brief, arterial blood samples were obtained from a carotid artery. The plasma samples were concentrated by centrifugation and serum was taken for determining LDH or CK activity.

Statistical Analysis —— Results are expressed as mean ± S.D. An analysis of variance (ANOVA) was performed to analyze the significance of intergroup differences. The significance of difference between two groups was analyzed by S-N-K test. Probability values < 0.05 were considered significant.

RESULTS

Effects of MI

Ligation of LAD resulted in severe changes in the ECG compared with in sham-operated controls. The average weight of the infarctions was 229.15 ± 23.98 mg, which was 41.6 ± 2.03% of left ventricular weight (Table 1). Plasma levels of LDH and CK were significantly increased in AMI rats ($p < 0.05$, Table 2). Furthermore, AMI resulted in increased MDA levels whereas TAOC and SOD levels decreased ($p < 0.05$, Table 3).

Observation of the FNL

Ibotenic acid as a neurotoxin has been used extensively to create lesions to gain insight into physiological control mechanisms.[8, 9] FNL by injection of the neurotoxin ibotenic acid conferred dramatic effects on posture and locomotion. To demonstrate that the neuronal bodies in the FN areas injected with ibotenic acid were effectively destroyed, we did a comparison. Almost all Nissl bodies were destroyed in the right FN areas where ibotenic acid was injected, whereas sections of the left FN where in saline was injected as control were normal (Fig. 1).

Effects of FNS

Electrical stimulation of FN reduced infarct

Table 1. Influence of FN Electrical Stimulation on Infarct Size in Rats

Group	No. (n)	Body weight (g)	Left ventricular weight (mg)	Infarct region weight (mg)	Infarct size (%)
Sham	10	285.50 ± 14.02	569.43 ± 50.13	0	0
AMI	9	289.44 ± 12.10	558.03 ± 46.29	229.15 ± 23.98	41.60 ± 2.03
FNS+AMI	11	280.90 ± 16.55	541.02 ± 40.30	135.80 ± 11.60*,**	25.16 ± 2.30*,**
FNL+FNS+AMI	10	284.50 ± 16.57	552.45 ± 48.10	225.17 ± 29.37$^{\#}$	40.39 ± 2.70$^{\#}$

$^{*}p < 0.01$ *vs.* AMI group, $^{**}p < 0.01$ *vs.* FNL+FHS+AMI group, $^{\#}p > 0.05$ *vs.* AMI group.

Table 2. Impact of FN Electrical Stimulation on Activities of Serum LDH and CK in MI Rats

Group	No. (n)	LDH ($U \cdot L^{-1}$)	CK ($U \cdot L^{-1}$)
Sham	10	954 ± 90	270 ± 40
AMI	9	1612 ± 123**	715 ± 110**
FNS+AMI	11	1056 ± 102*	373 ± 59*
FNL+FNS+AMI	10	1583 ± 139$^{\#}$	702 ± 95$^{\#}$

$^{*}p < 0.01$ *vs.* AMI group, $^{**}p < 0.01$ *vs.* Sham group, $^{\#}p > 0.05$ *vs.* AMI group

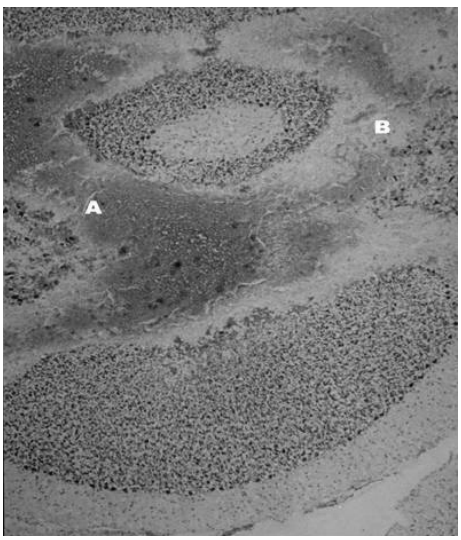

Fig. 1. Observation of the FN lesion

Nissl bodies almost disappeared in the right FN areas (A), but were normal in the left FN (B).

weight and size ($p < 0.05$, Table 1), and significantly lowered the activities of LDH and CK in comparison with the AMI group ($p < 0.01$, Table 2). There was a significant attenuation of MDA contents and marked increases of TAOC and SOD activities in the FNS group ($p < 0.01$, Table 3).

DISCUSSION

Myocardial ischaemia and/or necrosis in MI triggers inflammation and subsequent repair processes. Oxidative stress and chronic inflammatory responses play major roles in the initiation and progression of MI. MI increases concentrations of total plasma homocysteine. Possible mechanisms of accelerated vascular disease in hyperhomocysteinemia include endothelial cell injury, endothelial dysfunction, increased platelet adhesiveness, enhanced oxidative stress, and activation of the coagulation cascade.[10–12] Recently, many studies support a positive association between reactive oxygen species (ROS) and pathogenesis of myocardial ischaemia. Among the compounds that result from lipid peroxidation, MDA was widely looked upon as a marker of oxidative damage. Macrophages could take in oxidatively modified low density lipoprotein via scavenger receptor and transform into foam cells. Antioxidative material opposed the harmful effects of ROS. This beneficial scavenging function against superoxide was provided mainly by SOD. Thus SOD in the plasma may serve as an indicator of the balance between the damaging effects and the bioscavenging capability of superoxide.[13]

Oxidative damage is also referred to as OFR-induced damage. The present study demonstrated that linked reactions of OFR also play an important role in the pathogenesis of myocardial ischemia. Moreover, oxidative damage can cause continued damage to myocardium after infarction. MI is known to increase cytokine activity such as interleukin-1β, interleukin-6, and tumor necrosis factor-alpha, as well as catecholamine oxidation, both of which cause further myocardial damage.[14, 15] This starts a vicious cycle that further amplifies the increase of OFR and myocardial damage. Thus cardiomyocyte damage may be prevented and reversed if antioxidants are involved as soon as possible.

Circulating levels of enzymes such as CK and LDH are positively correlated with myocardial damage and MI size.[16] Increased plasma levels of CK may contribute to the pathogenesis of many dis-

Table 3. Effect of FN Electrical Stimulation on MDA Content and TAOC and SOD Activity in MI Rats

Group	No. (n)	MDA ($nmol \cdot mg^{-1} \cdot pro^{-1}$)	TAOC ($U \cdot mg^{-1} \cdot pro^{-1}$)	SOD ($nU \cdot mg^{-1} \cdot pro^{-1}$)
Sham	10	1.90 ± 0.14	1.32 ± 0.16	52.19 ± 7.07
AMI	9	$3.89 \pm 0.19^{**}$	$0.46 \pm 0.11^{**}$	$25.00 \pm 6.71^{**}$
FNS+AMI	11	$1.99 \pm 0.15^{*}$	$1.57 \pm 0.10^{*}$	$50.17 \pm 6.29^{*}$
FNL+FNS+AMI	10	$3.92 \pm 0.16^{\#}$	$0.51 \pm 0.11^{\#}$	$23.97 \pm 6.20^{\#}$

$^{*}p < 0.01$ *vs.* AMI group, $^{**}p < 0.01$ *vs.* Sham group, $^{\#}p > 0.05$ *vs.* AMI group.

orders. Possible atherogenic mechanisms include promotion of inflammation, cell adhesion and infiltration, and ROS generation.[17] In this study, we found that electrical stimulation of the FN decreased LDH and CK activities in serum. The decreased levels were likely a result of the reduced MI size. Lesioning of the FN prevented the cardioprotective effects of FN electrostimulation, suggesting that the FN played a crucial role in regulating these effects.

MDA is a lipid peroxidation product, the levels of which reflect the degree of tissue oxidative damage.[18] In this study, FNS reduced the stimulatory effects of MI on MDA content. TAOC and SOD reflect the ability to protect against OFR system. FNS markedly increased TAOC and SOD activity in FNS-treated rats. Furthermore, bilateral FN lesions prevented the protective effects of TAOC and SOD. These results indicate for the first time that the cardioprotective effects of FN electrical stimulation are associated with the suppression of oxidative damage. Previous studies have shown that FNS has neuroprotective effects against damage of ischemia.[19] One-hour electrical stimulation of FN can decrease infarction volume by 58% in persistent cerebral ischemia.[3] However, the exact mechanism of FNS-induced cerebral infarction decrease remains unclear. There are 3 potential mechanisms behind the protective effects of FN electrical stimulation: FNS suppressed the release of catecholamines,[20] thereby decreasing OFR by suppressing catecholamine oxidation; FNS inhibited the rate-limiting enzyme (cyclooxygenase) for arachidonic acid metabolism,[21] consequently inhibiting OFR; and FNS might suppress expression of nuclear transcription factor kappa-B (NF-κB) resulting in decreased OFR.[3]

It was reported that electrical stimulation of FN percutaneously in rats with local cerebral infarction had the similar effect as stimulation of FN directly.[22] The demonstration of similar effects of direct or indirect FNS implies that *in vitro* administration of percutaneous FNS could be of clinical relevance. It can be forecast that indirect exploration of FNS in cardiovascular diseases would be beneficial in the prevention and treatment of diseases. This needs to be confirmed by further study in the field.

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