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Myocardial infarction induces cognitive impairment by increasing the production of hydrogen peroxide in adult rat hippocampus

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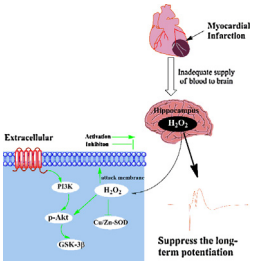
HIGHLIGHTS

- The long-term potentiation was suppressed in dentate gyrus area of MI rat model.
- NR2B expression was decreased in hippocampus of MI rats.
- H_2O_2 was significantly increased whereas the Cu/Zn-SOD activity was attenuated.
- PI3K/Akt pathway was activated in hippocampus of MI rats.

GRAPHICAL ABSTRACT



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ABSTRACT

Accumulating clinical evidence has shown a causal relationship between heart diseases and cognitive impairment in clinical, but the underlying mechanism remains unclear. In this study, rats with myocardial infarction (MI) were used to investigate cognition-related synaptic function and proteins. Adult male Wistar rats were subjected to MI by ligating of left anterior descending artery (LAD) and the infarct size of rat heart was measured by 2,3,5-triphenyltetrazoium chloride (TTC) staining. In this study, results showed that compared with control group, long-term potentiation was suppressed in dentate gyrus area, the contents of hydrogen peroxide (H_2O_2) and malondialdehyde were significantly increased, whereas the Cu/Zn-superoxide dismutase activity and N-methyl-D-aspartate receptor subunit 2B were attenuated in hippocampus of MI rats. Interestingly, it was observed that the PI3K/Akt pathway was activated in MI rats. Therefore, this study suggests that H_2O_2 plays an important role in cognitive dysfunction induced by MI.

1. Introduction

Heart diseases, especially myocardial infarction (MI) are one of major threats to public health in both developed and developing countries [1]. When MI occurred, the blood supplied to the heart is decreased, then reactive oxygen species (ROS) are produced [2]. ROS, particularly hydrogen peroxide (H_2O_2), possess highly reactive and toxic properties and are able to damage several proteins, lipids and DNA [2,3]. Antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD), are able to antagonize the effect of reactive species, but all of them are decreased after MI [3].

Meanwhile, heart diseases not only decrease blood flow to the heart, but also reduce blood flow to other organs, such as brain [4]. Now there is increasing evidence to suggest that heart diseases may be a factor leading to the progress of cognitive impairment [5]. The cognitive dysfunction is relatively common in patients with chronic congestive heart failure [5,6]. When patients administrated with digoxin, a drug used to cure congestive heart failure in clinical, the cognitive performance will be ameliorated [6]. The inadequate supply of blood leads to the deficiency of oxygen and nutrients in brain, then ROS would be produced and cause the cell damage in many

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cerebral areas including the hippocampus. As known, hippocampus plays a pivotal role in learning, memory and other cognitive abilities and is sensitive to ischemia [7]. Studies have shown that ROS, particularly H_2O_2, are able to modulate long-term potentiation (LTP) [8]. Millimolar concentrations of H_2O_2 irreversibly suppress the synaptic transmission and the plasticity [8]. Although studies have indicated a positive correlation between heart disease and cognitive impairment in clinical [5,6], a rat model to study the underlying mechanism of the cognitive impairment induced by MI is so far missing. In this study, we ligated the rat left anterior descending coronary artery to induce MI rat model and aimed to investigate the effects of the heart disease on cognitive performance, and their underlying mechanisms.

2. Materials and methods

2.1. Animal care

Adult male Wistar rats (250–300 g) were used in this experiment. Rats were housed at specific-pathogen free condition with free access to food and water under a 12 h light/dark cycle with lights on from 8 a.m. All animal experiments were performed in accordance with the Animal Management Rules of the Ministry of Health of the People's Republic of China and approved by the Animal Research Ethics Committee, School of Medicine, Nankai University.

2.2. Induction of myocardial infarction

Adult male Wistar rats (250–300 g) were randomly assigned into two groups: control group ($n = 6$) and myocardial infarction (MI) group ($n = 12$). The rat model of MI was established as previously described [9]. Briefly rats were anesthetized by injecting 7% chloral hydrate (35 mg/kg) intraperitoneally, then the left anterior descending coronary artery was ligated with a 6-0 polypropylene suture around 1–2 mm distal to the left auricle in MI group. For control group, all operative procedures were performed identically, except for coronary artery ligation. Finally, heart samples were collected 6 h after MI, washed in ice-cold phosphate buffer saline and stored at −20 °C for infarct size measurement. The hippocampi were collected 6 h after MI and stored at −80 °C for protein determination until used.

2.3. Infarct size measurement

The myocardial infarct size was measured by 2,3,5-triphenyltetrazoium chloride (TTC) staining as previously reported [9]. Briefly, the rat heart ($n = 6$ per group) was cut into five coronal slices ($n = 5$ per group), incubated in 1% (w/v) TTC solution for 15 min at 37 °C, then fixed in 4% paraform for another 30 min. The infarct size was defined as a ratio of the left ventricular (LV) infarct area to the whole LV area [3]. The size of the infarct area and the total LV area were calculated by a computer using the software of Scion Image.

2.4. Long-term potentiation recording

The long-term potentiation (LTP) recording was performed on rats immediately before the collection of hearts. LTP recording was performed as previously described [10]. Rats ($n = 6$ per group) were anesthetized with 7% chloral hydrate (35 mg/kg), positioned on a stereotaxic frame (SR-6N; Narishige, Japan). Recordings of the field excitatory post-synaptic potentials (fEPSPs) were made from the perforant pathway (PP) to dentate gyrus (DG) region. Firstly, the bipolar stimulating electrode was lowered into the PP area of the left cerebral hemisphere (coordinates: 8.0 mm posterior to bregma, 4.4 mm lateral to the midline, and approximately 2.8–3.8 mm ventral to the cortical surface). Then the recording electrode was lowered into DG area of the left cerebral hemisphere (coordinates: 4.2 mm anterior to bregma, 2.5 mm lateral to the midline, approximately 3.0–3.7 mm ventral to the cortical surface) [10]. The baseline responses were recorded for 20 min after the stable curves were obtained. Finally, theta burst stimulation (TBS) protocol was used to induce a potentiation of the synaptic response. The protocol consisted of four theta epochs delivered at 0.1 Hz. Each epoch, in turn, consisted of 20 trains of four pulses (at 200 Hz) delivered at 5 Hz with the same stimulus intensity as the baseline pulse. The amplitude of fEPSPs would be recorded for a further period of 60 min.

2.5. Measurements of H_2O_2, malondialdehyde (MDA) and superoxide dismutase (SOD)

The levels of H_2O_2, MDA and SOD were measured ($n = 6$ per group) using their kits (Biotime Biotech Haimen, China) according to the manufacture's protocol.

2.6. Western blotting assay

The Western blotting assay was performed as previously described [9,11]. The rat hippocampus ($n = 4$ per group) was separated from brain, homogenized in lysis buffer (Beyotime Biotechnology, Haimen, China), centrifugated at 12,000 rpm for 10 min at 4 °C, and the supernatant was collected and stored at −80 °C. The concentration of protein was detected by enhanced BCA protein assay kit (Beyotime Biotechnology, Haimen, China). Forty micrograms of the protein lysates were separated on SDS-PAGE gels, and then transferred onto 0.45-μm polyvinylidene fluoride (PVDF) membrane (Promega Co. Ltd.). Thirdly, the PVDF membrane was blocked in Tris-buffered saline with Tween-20 (TBST) containing 5% skim milk for 1.5 h at room temperature and probed with rabbit anti-rat NR2A and NR2B (1:4000, abcam, UK), β-actin (abcam, UK, 1:4000), Akt (1:2000, Cell Signaling Technology, USA) and p-Akt (ser437, 1:2000, Cell Signaling Technology, USA), antibody diluted in TBST containing 5% skim milk overnight at 4 °C. Finally anti-rabbit IgG (1:8000, abcam, UK) diluted in TBST was used as secondary antibody. Protein band intensities were quantified by using a Western blotting detection system.

2.7. Statistical analysis

Data were presented as means ± S.E.M and analyzed by SPSS software. Student's test (two-tailed) was used to test the significance of differences between mean values. Mean values are given with standard errors of the mean. Pictures were processed with Photoshop software. Differences at $P < 0.05$ were considered statistically significant.

3. Results

3.1. Ligating left anterior descending coronary artery induced MI

To test if the rat model of MI was successfully duplicated, TTC staining was performed. Our results showed that the non-ischemic myocardial tissue stained bright red, whereas the ischemic myocardial tissue remained white (Fig. 1A). The infarct size was 50.25 ± 1.85% in MI group in this experiment (Fig. 1B), which indicated that the surgical procedure was effective and sufficient to duplicate the MI model in rats. In this study, twelve rats were suffered coronary artery ligation. Unfortunately, four rats died, eight