Effect of Hydrogen on the Drp 1-dependent Mitochondria after Brain I/R

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Abstract

Objective: To evaluate the effect of hydrogen on the apoptotic pathway of Drp 1-dependent mitochondria division after brain I/R. Methods: 60 clean-grade male SD rats (200-220g) were divided into 3 groups by random numbers, blank (n=20); I/R (n=20) and I/R + H2 (n=20). Rat brain I/R injury model was made by the line-bolt method. Group I/R + H2 was injected with hydrogen intraperitoneally 1h and 6h, respectively. Groups I/R and I/R + H2 were severed under anesthesia after successful surgery, and the cerebral infarction area was observed by TTC staining. SD rats were killed at 6, 12, and 24h after surgery, and the expression of mitochondrial motility-related protein 1 (Drp 1) was determined in the brain tissue by Western blot. Results: The TTC staining showed that the treatment with the I/R rats reduced the infarction area of cerebral ischemia and reperfusion. Drp 1 expression was up-regulated in I/R and I/R + H2 groups (P <0.05); Drp 1 expression was downregulated in I/R + H2 groups compared with I/R groups (P <0.05). Conclusion: The mechanism by which hydrogen gas improves the mitochondrial function in I/R rats may be related to its promotion of mitochondrial fusion and the inhibition of mitochondrial fission.

Keywords

Hydrogen, I/R, Drp 1, and mitochondria

Ischemic stroke is characterized by insufficient blood supply to the brain, brain tissue hypoxia and glucose deficiency, followed subsequent destructive and irreversible brain damage [1, 2]. There are 795,000 patients with new or recurrent stroke annually, about 87% of whom are ischemic stroke [3]. Besides the resection, the tissue prothrombin activator (t-PA) remains the only FDA-approved drug used for the reconstruction of the ischemic brain blood supply. Blood supply to the only FDA approved drug, but with a 4.5-hour prime treatment time limit. Delayed thrombolytic therapy carries the risk of hemorrhagic transformation and triggers multiple complications, neurodegeneration [4], and neuronal damage, resulting in clinically less than 10% of patients with ischemic stroke receiving t-PA [5]. Developing new therapeutic strategies to reduce ischemia-reperfusion injury is very important for protecting the surviving brain tissue of ischemic stroke patients [6, 7].

Autophagy is a process of "autofagy" that removes aggregated proteins or damaged organelles through degradation in the autolyase body. Basal autophagy is indispensable for cell survival, and excessive autophagy may be detrimental to during cerebral ischemia-reperfusion injury [8]. Mitochondria are key regulators of cellular energy homeostasis in response to physiological adaptations and stress conditions [9]. Mitophagy, called mitophagy, has a dual role in ischemic stroke [10, 11]. Mitosis is a critical for the control of mitochondrial quality and quantity under physiological conditions. In contrast [2, 12], inhibition of mitochondrial fragmentation/mitophagy also showed a neuroprotective effect on ischemic brain injury [10, 13]. Recent studies have shown that reperfusion after cerebral
ischemia is a critical turning point in autophagy/mitochondria progression from a protective mechanism to a destructive program [14, 15]. In this process, the mitochondrial recruitment of dynamin-associated protein 1 (Drp 1) and PINK 1/Parkin plays a key role in the activation of mitophagy during cerebral ischemia-reperfusion injury, [16-18]. Excessive expression of PINK 1 and Parkin was found to co-localize [19] with LC3-positive vesicles. The ubiquitin kinase PINK 1 phosphorylates ubiquitin and activates the ubiquitin ligase. The ubiquitin ligase Parkin, which recruits autophagic receptors and triggers mitotic phagocytosis. Activation of the Drp 1/PINK 1/Parkin signaling pathway mediates mitosis and contributes to brain injury [20, 21] in cerebral ischemia and reperfusion injury. Thus, the Drp 1/PINK 1/Parkin signaling pathway is an important therapeutic target to combat cerebral ischemia-reperfusion injury.

Hydrogen is a new type of therapeutic substance, used mainly in water or gaseous solutions. It has been shown to be effective against cancer, lung injury, skin tumors, and liver injury and it has anti-inflammatory, anti-oxidation, and anti-apoptotic effects [22-25]. However, the study of the action or mechanism of molecular hydrogen on IRI is still lacking, although some studies have revealed the molecular mechanism of hydrogen therapy [23, 26, 27]. Therefore, in this study, we aimed to determine the role of hydrogen treatment in the rat I/R model in preventing long-term injury and recovery from transient injury, and suggest a molecular pathway of hydrogen on Drp 1-dependent mitochondrial fission apoptosis in cerebral ischemia-reperfusion injury.

1. Materials and Methods

1.1 Animal selection and grouping

60 (200-220g) in 3 groups by random number chart, blank group (n=20); I/R group (n=20); and I/R + H2 group (n=20).

1.2 Establishment of the rat I/R model

Water was fasting for 12h before surgery. Rat brain I/R injury model was made by the line-bolt method. Rats were anesthetized with 4% chloral hydrate (1 ml of 4% chloral hydrate per 100 g of body weight). After anesthesia, the rats were fixed in the supine position on the operating table, and the skin was cut at 0.5 cm near the middle of the neck. After exposing the left common carotid artery, the internal and external carotid arteries were separated. The root of the left external carotid artery was ligated with a surgical line, and the hemostasis clamp temporarily clamped the left cervical sum and the internal carotid artery. A small mouth was cut at the near bifurcation of the external carotid artery, and the plug clamp was inserted after releasing the plug thread, and the thread was tightened and fixed the thread to embolize the middle cerebral artery. After 90 min of embolization, the plug line was removed to the stump of the external carotid artery for reperfusion for 24 h. Sham group except for no bolt insertion, other operation as above. The H2 group received hydrogen intraperitoneal injection according to 10 ml/kg dose standard when pulling out the plug line, Sham group and Mod group, air intraperitoneal injection at 10 ml/kg as a control, and then every 12 h injection (three injections). During the experiment, the temperature in the controlled room was around 37℃, and the rats were insulated with an electric heating plate until they resumed activity. At 24 h after successful experimental animal model preparation, the rats were anesthetized using 4% chloral hydrate, and the skin was cut open from the abdomen, and the heart was exposed. The left atrium was punctured to the left ascending aorta with a 50 ml syringe and the left a trial appendage was cut open and perfused with ice-cold saline. After perfusion, the brain tissue was removed, and the left cerebral cortex was removed transferred to a frozen storage tube. It was flash-frozen in liquid nitrogen and then frozen in a-80℃ refrigerator for protein and mitochondrial extraction. Brain tissue used for paraffin sections was infused in ice-cold saline followed with 4% paraformaldehyde, and the removed brain tissue was fixed in 4% paraformaldehyde.

1.3 Preparation and use of hydrogen gas

The Jinan Haowei pure water hydrogen generator is used to produce hydrogen gas, and pure water is added to the instrument. Electrolytic pure water can produce hydrogen gas. After electrification, the cathode of the electrolytic cell produces hydrogen, the anode produces oxygen, and the hydrogen enters the hydrogen/water separator. Oxygen is discharged into the atmosphere through the outlet. The hydrogen/water separator separates the hydrogen from the water. After entering the dryer and dehumidifier, the hydrogen is output from the outlet after the pressure regulator valve and regulating valve. After electrolysis of pure water, the ratio of hydrogen and oxygen is 2:1. The purity of the hydrogen produced can be more than 99%. The hydrogen was collected in a 10 ml syringe and used for intrape-
ritoneal injection.

1.4 Cerebral infarct area was visualized by TTC staining

After the animal model, the brain was severed under anesthesia. After removing the brain tissue, immediately put it into the-20°C refrigerator, freeze it for 30 min, then remove the 2 mm coronal plane and cut it into 5 pieces. The middle three pieces of the sections in a dish containing 2,3,3,5-chloride tetrazolole staining solution were incubated for 20 min, and the sections were turned at 10 min intervals. The sections were counterstained and then fixed with 4% paraformaldehyde for 24 h. After fixation, it was placed in order, photographed with the camera and the percentage of infarct area was calculated using Image J software.

1.5 Extraction of mitochondria from ischemic neural cells in cortical areas

Follow the instructions of the mitochondrial extraction kit (Solebo). Weigh 200 mg of fresh brain tissue, rinsed with PBS, washed blood and blotted dry with filter paper. The brain tissue was cut with scissors and placed into a small-volume glass homogenizer. 1.0 ml of ice pre-chilled Lysis Buffer was added and the brain tissue was ground on ice 20 times. Brain homogenates were transferred to a 2 ml centrifuge tube and centrifuged for 5 min at 4°C at 1000 g. The supernatant was removed and transferred to a new centrifuge tube and centrifuged again for 5 min at 4°C at 1000 g. The supernatant was removed and transferred to a new centrifuge tube at 4°C at 12000 g for 10 min at a centrifugation force. The supernatant was transferred to a new centrifuge tube, and protein was extracted and quantified to determine mitochondrial concentration. Mitochondria were precipitated at the bottom of a centrifuge tube, and 0.5 ml Wash Buffer resuspended mitochondria was added to the precipitate and then centrifuged again for 5 min under 4°C at 1000 g. The supernatant was removed and transferred to a new centrifuge tube and centrifuged at 12000 g for 10 min. The supernatant was removed, and highly purity mitochondria were precipitated at the bottom of the tube. Mitochondria were resuspended in 50 ul Store Buffer and stored in a-80°C refrigerator.

Statistical processing was analyzed by SPSS13.0 software, normally distributed measurement data were expressed as mean ± standard deviation (X ± s), one-way analysis of variance, and P <0.05 was considered statistically significant.

2. Results

1) Hydrogen can reduce the cerebral infarction area and improve the neural function in rats with cerebral ischemia and reperfusion.

TCC staining showed that compared with the cerebral infarct area of rats in I/R group, the infarct area of cerebral ischemia and reperfusion was reduced by intraperitoneal hydrogen injection of hydrogen (see Fig. 1).
2) Drp 1, was up-regulated in I/R and I/R + H2 (P <0.05), and Drp 1 was downregulated in I/R + H2 versus I/R (P <0.05) in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>6h</th>
<th>12h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>blank group</td>
<td>0.291±0.051</td>
<td>0.273±0.044</td>
<td>0.342±0.022</td>
</tr>
<tr>
<td>I/R group</td>
<td>0.915±0.032a</td>
<td>0.931±0.041a</td>
<td>0.952±0.043a</td>
</tr>
<tr>
<td>I/R+H2 group</td>
<td>0.594±0.041ab</td>
<td>0.632±0.053ab</td>
<td>0.654±0.062ab</td>
</tr>
</tbody>
</table>

Note: a P <0.05 versus blank group and b P <0.05 versus I/R group

3. Discussion

The preliminary study of this topic showed that the inhalation of hydrogen for 1h and 6h respectively for 1h could effectively improve the survival rate of I/R rats, improve organ function and play an organ protective role [28-30]. Therefore, this method was used for hydrogen treatment in this study, and the results showed that the I/R + H2 rats were effectively reduced after surgery compared with the I/R group.

Mitochondria produce ATP through oxidative phosphorylation, and the premise of this process is that the mitochondria establish an electrochemical gradient known as MMP [15] through oxidative respiration. As a key protein in regulating mitochondrial fission, Drp 1 plays a crucial role in maintaining the number, size, shape, and function of mitochondria. Therefore, in this study, Western blot was used to determine Drp 1 expression levels in brain tissue to react to mitochondrial dynamics. The results of this study showed that Drp 1 expression in I/R group was up-regulated compared with the blank group, suggesting that mitochondrial function was impaired, mitochondrial fission increased and fusion decreased; compared with I/R group, Drp 1 expression was downregulated in I/R + H2 group, suggesting that hydrogen could promote mitochondrial fusion and inhibit mitochondrial fission, thus improving the function of mitochondrial body in the brain tissue of I/R rats.

Fund Project

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References


