Effects of sevoflurane pretreatment on cerebral ischemia reperfusion injury in mice

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ARTICLE INFO

ABSTRACT

Objective: To study the effects of sevoflurane pretreatment on cerebral ischemia reperfusion injury in mice. Methods: C56 BL/6J mice were selected as experimental animals and divided into control group, I/R group and Sev group. I/R group and Sev group were established into cerebral ischemia reperfusion injury models through suture method, and Sev group were given consecutive 4 days of sevoflurane pretreatment before model establishment. The ischemia reperfusion brain tissue was collected to determine the mRNA expression of apoptosis genes and the levels of oxidative stress indexes. Results: Bcl-2, HAX-1, Mcl-1 and Survivin mRNA expression as well as Prdx6 and SOD levels in brain tissue of I/R group were significantly lower than those of control group whereas NF-kB, p53, PTEN and Fas mRNA expression as well as Nox4, ROS and MDA levels were significantly higher than those of control group; Bcl-2, HAX-1, Mcl-1 and Survivin mRNA expression as well as Prdx6 and SOD levels in brain tissue of Sev group were significantly higher than those of I/R group whereas NF-kB, p53, Noxa, PTEN and Fas mRNA expression as well as Nox4, ROS and MDA levels were significantly lower than those of I/R group. Conclusion: Sevoflurane pretreatment can inhibit the apoptosis and oxidative stress response to alleviate the cerebral ischemia reperfusion injury in mice.

1. Introduction

Ischemic cerebrovascular diseases are the common diseases in clinic, and local tissue ischemia can directly cause hypoxic injury. In recent years, with the progress of interventional treatment and thrombolytic treatment means, a growing number of patients with ischemic cerebrovascular disease can get timely reperfusion therapy, but under the influence of ischemia-reperfusion injury, some patients will still get varying degrees of neurological damage after reperfusion therapy. In the process of cerebral ischemia reperfusion, the apoptosis and oxidative stress over-activation are the important pathological links in local tissue damage, and the anti-apoptosis and anti-oxidation are the important targets to reduce or prevent ischemia-reperfusion injury at present[1-2]. Sevoflurane is an inhalational anesthetic widely used in clinic, recent studies have confirmed that sevoflurane can protect the ischemia-reperfusion injury process in myocardial tissue, lung tissue and nerve tissue[3,4], but it is still not clear about the specific molecular mechanisms of sevoflurane to protect the cells. In the following studies, we analyzed the effects of sevoflurane pretreatment on cerebral ischemia reperfusion injury in mice from the perspectives of apoptosis and oxidative stress response.

2. Experimental materials and methods

2.1 Experimental materials

The experimental animals were C56 BL/6J mice, which weighed 20-26 g, were provided by the Guangdong Medical Laboratory Animal Center, were under the license number SCXK (Guangdong) 2008-0002, were approved by the hospital ethics committee and then used for experimental study. The kits for RNA separation, cDNA synthesis and PCR reaction were from Takara Company,
radioimmunoprecipitation kits were purchased in Nanjing Jiancheng Institute, and ROS kits were purchased in Shanghai Beyotime Company.

2.2 Experimental methods

2.2.1 Model establishment and intervention

The experimental animals were divided into control group, I/R group and Sev group. I/R group and Sev group were established into cerebral ischemia reperfusion models according to the following method: after intraperitoneal injection of chloral hydrate for anesthesia, the right common carotid artery and external carotid artery were separated and ligatured, then a small opening was cut in the bifurcation of common carotid artery, internal carotid artery and external carotid artery, a special suture with diameter 0.3 mm was inserted, inserting depth was better 17-20 mm from the bifurcation, middle cerebral artery blood flow was blocked for 30 min, and then the blood clots were taken out to complete reperfusion. Sev group were given sevoflurane pretreatment before model establishment, and the method was as follows: the mice were placed in the anesthesia respiratory box with 2.4% sevoflurane/air and breathed freely for 30 min each time, 1 time a day for four consecutive days; before model establishment, control group and I/R group were also placed in the anesthesia respiratory box and given 100% air inhalation.

2.2.2 Gene mRNA expression detection

The right amount of ischemia-reperfusion brain tissue was collected, cDNA kit was used to separate and extract RNA and synthesize it into cDNA, then PCR kits as well as the specific primers for Bcl-2, HAX-1, Mcl-2, Survivin, NF-kB, p53, PTEN and Fas gene were used for reaction, and the reaction curve was referred to calculate the gene mRNA expression.

2.2.3 Oxidative stress index content detection

The right amount of ischemia-reperfusion brain tissue was collected, added in PBS and fully cracked, the obtained tissue suspension was centrifuged to abandon the precipitation and keep the supernatant, radioimmunoprecipitation kits were used to detect Prdx6, SOD, Nox4 and MDA contents, and ROS kits were used to detect the ROS contents.

2.3 Statistical methods

SPSS 19.0 software was used to input data, the measurement data among three groups were by variance analysis and \( P < 0.05 \) indicated that the differences in analysis results were statistically significant.

3. Results

3.1 Apoptosis–related gene expression in brain tissue

Bcl-2, HAX-1, Mcl-1 and Survivin mRNA expression in brain tissue of I/R group were significantly lower than those of control group whereas NF-kB, p53, PTEN and Fas mRNA expression were significantly higher than those of control group; Bcl-2, HAX-1, Mcl-1 and Survivin mRNA expression in brain tissue of Sev group were significantly higher than those of I/R group whereas NF-kB, p53, Noxa, PTEN and Fas mRNA expression were significantly lower than those of I/R group. Differences in pair-wise comparison of Bcl-2, HAX-1, Mcl-1, Survivin, NF-kB, p53, Noxa, PTEN and Fas mRNA expression in brain tissue were statistically significant among three groups of mice (\( P < 0.05 \)).

3.2 Oxidative stress index levels in brain tissue

Prdx6 (ng/mL) and SOD (U/mL) levels in brain tissue of I/R group were significantly lower than those of control group whereas Nox4 (U/mL), ROS (U/mL) and MDA (μmol/mL) levels were significantly higher than those of control group; Prdx6 and SOD levels in brain tissue of Sev group were significantly higher than those of I/R group whereas Nox4, ROS and MDA levels were significantly lower than those of I/R group.

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Bcl-2</th>
<th>HAX-1</th>
<th>Mcl-2</th>
<th>Survivin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>1.04±0.18</td>
<td>1.02±0.15</td>
<td>1.06±0.11</td>
<td>0.98±0.15</td>
</tr>
<tr>
<td>I/R</td>
<td>10</td>
<td>0.32±0.06*</td>
<td>0.26±0.05*</td>
<td>0.39±0.07*</td>
<td>0.23±0.04*</td>
</tr>
<tr>
<td>Sev</td>
<td>10</td>
<td>0.69±0.09*</td>
<td>0.58±0.07*</td>
<td>0.71±0.10*</td>
<td>0.60±0.08*</td>
</tr>
</tbody>
</table>

\*: compared with control group, \( P < 0.05 \);

Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>NF-kB</th>
<th>p53</th>
<th>Noxa</th>
<th>PTEN</th>
<th>Fas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>1.02±0.17</td>
<td>1.01±0.15</td>
<td>0.97±0.15</td>
<td>1.05±0.18</td>
<td>1.02±0.14</td>
</tr>
<tr>
<td>I/R</td>
<td>10</td>
<td>3.29±5.85*</td>
<td>2.77±0.42*</td>
<td>2.19±0.35*</td>
<td>2.91±0.46*</td>
<td>3.33±0.52*</td>
</tr>
<tr>
<td>Sev</td>
<td>10</td>
<td>1.77±0.20*</td>
<td>1.51±0.19*</td>
<td>1.42±0.20*</td>
<td>1.63±0.29*</td>
<td>1.71±0.27*</td>
</tr>
</tbody>
</table>

\*: compared with control group, \( P < 0.05 \); a: compared with I/R group, \( P < 0.05 \).
mediated by it the activity of various Caspase molecules during apoptosis cascade inhibitory effect on Caspase-3 activity, and the latter can inhibit apoptotic proteins widespread in the cells, the former has a direct is the inhalational anesthetic that protects the tissue organs tissue injury. It has been confirmed in recent years that sevoflurane molecules in the brain tissue to cause excessive apoptosis and brain injury showed that the Bcl-2, HAX-1, Mcl-1 and Survivin mRNA expression significantly reduced in brain tissue of I/R group. It shows in the process of excessive apoptosis in brain tissue. The Bcl-2 and Mcl-1 are the Bcl-2 family members with anti-apoptotic activity, which can form heterodimers with various pro-apoptosis molecules Bax and Bad and antagonize their pro-apoptotic activity to realize the inhibiting effect on apoptosis[5,6]; HAX-1 and Survivin are anti-apoptotic proteins widespread in the cells, the former has a direct inhibitory effect on Caspase-3 activity, and the latter can inhibit the activity of various Caspase molecules during apoptosis cascade reaction, and inhibit the Caspase activity to inhibit the apoptosis mediated by it[7]. In the study, analysis of the changes in above anti-apoptosis molecule expression in the process of ischemia reperfusion injury showed that the Bcl-2, HAX-1, Mcl-1 and Survivin mRNA expression significantly reduced in brain tissue of I/R group. It shows that ischemia-reperfusion can inhibit the expression of anti-apoptotic molecules in the brain tissue to cause excessive apoptosis and brain tissue injury. It has been confirmed in recent years that sevoflurane is the inhalational anesthetic that protects the tissue organs[8], the effect of sevoflurane on the anti-apoptotic molecule expression in the process of ischemia reperfusion was further analyzed in the study to reflect the neuroprotective effect of sevoflurane, and the results showed that Bcl-2, HAX-1, Mcl-1 and Survivin mRNA expression in brain tissue of Sev group were significantly higher than those of I/R group. This shows that sevoflurane can increase the expression of anti-apoptotic molecules in brain tissue during ischemia reperfusion process, and then enhance the anti-apoptotic capacity to reduce the ischemia reperfusion injury of brain tissue. The occurrence of apoptosis involves the abnormality of both anti-apoptotic links and pro-apoptotic links. In addition to the anti-apoptotic molecules whose expression levels change, a variety of pro-apoptotic molecules also change in expression. NF-kB is an important transcription factor that regulates apoptosis process, it is combined with inhibitor IkB and in the resting state under physiological conditions, the outside stimuli such as hypoxia and ischemia-reperfusion will cause the IkB phosphorylation and dissociation with NF-kb, and the free NF-kB enter into the nucleus and regulate the expression of various pro-apoptotic target genes[9]. p53 is one of the target genes regulated by NF-kB. On the one hand, it can antagonize the activity of various apoptosis proteins and inhibit cell proliferation to indirectly induce apoptosis[10]; on the other hand, it can enhance the function of Noxa, PTEN and other pro-apoptotic molecules and promote apoptosis[11,12]. Analysis of the changes in above pro-apoptosis molecule expression during ischemia reperfusion injury in the study showed that NF-kb, p53, Noxa, PTEN and Fas mRNA expression in the brain tissue of I/R group increased significantly. This indicates that ischemia reperfusion can increase the expression of pro-apoptotic molecules in the brain tissue, and then result in excessive apoptosis and brain tissue injury. Further analysis of the effects of sevoflurane on the pro-apoptotic molecule expression in ischemia reperfusion process in the study showed that NF-kb, p53, Noxa, PTEN and Fas mRNA expression in the brain tissue of Sev group were significantly lower than those of I/R group. This shows that sevoflurane can reduce the expression of pro-apoptotic molecules in brain tissue during ischemia reperfusion process, and then reduce ischemia reperfusion injury in brain tissue by inhibiting apoptosis. Oxidative stress is another important factor that causes tissue damage during ischemia reperfusion process, and it is mainly characterized by excessive generation of oxygen free radicals[13]. Nox family is the important metabolic enzymes that catalyze the generation of oxygen free radicals, and Nox4 content is the most abundant in the brain[14]; hypoxia and ischemia reperfusion will increase the expression and catalytic activity of Nox4, then increase the ROS production and cause oxidative damage to the tissues[15]. MDA is produced after the lipid in local tissue and cells reacts with ROS, and MDA is massively generated in the process of ROS-induced lipid peroxidation and tissue damage. A variety of antioxidant enzymes in local tissue play a certain oxygen free radical scavenging role in the process of oxidative stress reaction, which can avoid excessive oxidative damage to the tissue. SOD is an important antioxidant enzyme in the body, which can reduce the ROS with strong oxidation to the H2O2 with weak oxidization and reduce the oxidative damage from ROS to tissues[16,17]; Prdx6

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Prdx6</th>
<th>SOD</th>
<th>Nox4</th>
<th>ROS</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>10</td>
<td>1.84±0.24</td>
<td>22.58±3.96</td>
<td>3.28±0.52</td>
<td>2.51±0.38</td>
<td>10.29±1.84</td>
</tr>
<tr>
<td>I/R group</td>
<td>10</td>
<td>0.51±0.08</td>
<td>10.28±1.77</td>
<td>8.29±1.15</td>
<td>9.19±1.25</td>
<td>35.83±4.95</td>
</tr>
<tr>
<td>Sev group</td>
<td>10</td>
<td>1.05±0.19</td>
<td>16.58±2.52</td>
<td>5.48±0.79</td>
<td>4.51±0.58</td>
<td>17.40±2.48</td>
</tr>
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*: compared with control group, P<0.05; #: compared with I/R group, P<0.05.

4. Discussion

Ischemia reperfusion injury is an important factor that affects the efficacy of reperfusion therapy for ischemic cerebrovascular diseases. Oxidative stress and apoptosis are the pathological links closely related to ischemia reperfusion injury. The expression of various anti-apoptotic molecules has been significantly inhibited in the process of excessive apoptosis in brain tissue. The Bcl-2 and Mcl-1 are the Bcl-2 family members with anti-apoptotic activity, which can form heterodimers with various pro-apoptosis molecules Bax and Bad and antagonize their pro-apoptotic activity to realize the inhibiting effect on apoptosis[5,6]; HAX-1 and Survivin are anti-apoptotic proteins widespread in the cells, the former has a direct inhibitory effect on Caspase-3 activity, and the latter can inhibit the activity of various Caspase molecules during apoptosis cascade reaction, and inhibit the Caspase activity to inhibit the apoptosis mediated by it[7]. 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This shows that sevoflurane can increase the expression of anti-apoptotic molecules in brain tissue during ischemia reperfusion process, and then enhance the anti-apoptotic capacity to reduce the ischemia reperfusion injury of brain tissue. The occurrence of apoptosis involves the abnormality of both anti-apoptotic links and pro-apoptotic links. In addition to the anti-apoptotic molecules whose expression levels change, a variety of pro-apoptotic molecules also change in expression. NF-kB is an important transcription factor that regulates apoptosis process, it is combined with inhibitor IkB and in the resting state under physiological conditions, the outside stimuli such as hypoxia and ischemia-reperfusion will cause the IkB phosphorylation and dissociation with NF-kb, and the free NF-kB enter into the nucleus and regulate the expression of various pro-apoptotic target genes[9]. p53 is one of the target genes regulated by NF-kB. On the one hand, it can antagonize the activity of various apoptosis proteins and inhibit cell proliferation to indirectly induce apoptosis[10]; on the other hand, it can enhance the function of Noxa, PTEN and other pro-apoptotic molecules and promote apoptosis[11,12]. Analysis of the changes in above pro-apoptosis molecule expression during ischemia reperfusion injury in the study showed that NF-kb, p53, Noxa, PTEN and Fas mRNA expression in the brain tissue of I/R group increased significantly. This indicates that ischemia reperfusion can increase the expression of pro-apoptotic molecules in the brain tissue, and then result in excessive apoptosis and brain tissue injury. Further analysis of the effects of sevoflurane on the pro-apoptotic molecule expression in ischemia reperfusion process in the study showed that NF-kb, p53, Noxa, PTEN and Fas mRNA expression in the brain tissue of Sev group were significantly lower than those of I/R group. 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Table 3.
Oxidative stress index levels in brain tissue of three groups of mice.
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is an antioxidant enzyme that takes the glutathione and ascorbic acid as electron donor, which can remove the ROS and H₂O₂ and suppress the excessive activation of oxidative stress response[18]. The analysis of the changes in oxidative stress indexes and the regulating effect of sevoflurane in the process of ischemia reperfusion in the study showed that Prdx6 and SOD contents in brain tissue of I/R group decreased significantly whereas Nox4, ROS and MDA contents increased significantly; Prdx6 and SOD contents increased significantly whereas Nox4, ROS and MDA contents decreased significantly after sevoflurane treatment. This indicates that the oxidative stress response is significantly activated during the cerebral ischemia reperfusion process, and sevoflurane can inhibit the oxidative stress response and reduce injury in ischemia reperfusion process.

The apoptosis and oxidative stress are significantly activated during the cerebral ischemia reperfusion injury; sevoflurane pretreatment has protective effect on cerebral ischemia reperfusion injury, and inhibiting apoptosis and oxidative stress response is its molecule pathway to exert protective effect.

References


