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Study on cerebral edema in the rat model with closed craniocerebral injury and its relationship with apoptosis, inflammatory factor generation and AQPs expression
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ABSTRACT

Objective: To study the relationship of cerebral edema with apoptosis, inflammatory factor generation and AQPs expression in the rat model with closed craniocerebral injury. Methods: Male SD rats were selected as experimental animals and divided into model group and control group. Model group were established into the closed craniocerebral injury models and control group received sham operation. The water content of damaged brain tissue as well as the expression of apoptosis molecules, the generation of inflammatory factors and the expression of aquaporins (AQPs) molecules were measured 7 d after model establishment. Results: The water content of brain tissue of model group was significantly higher than that of control group; Homer1a, Pim-3, Bcl-2, AQP1, AQP4 and AQP9 mRNA expression in brain tissue of model group were significantly lower than those of control group while Cdk5, FasL and Caspase-3 mRNA expression as well as NF-kB, TNF-α, IL-1β, IL-6 and p-JNK generation were significantly higher than those of control group. Homer1a, Pim-3, Bcl-2, AQP1, AQP4 and AQP9 mRNA expression in brain tissue of model group were negatively correlated with water content while Cdk5, FasL and Caspase-3 mRNA expression as well as NF-kB, TNF-α, IL-1β, IL-6 and p-JNK generation were positively correlated with water content. Conclusion: The excessive apoptosis, increased inflammatory factor generation and decreased AQPs expression are closely related to the occurrence of cerebral edema in the process of closed craniocerebral injury.

1. Introduction

Craniocerebral trauma is a type of trauma with high fatality rate and disability rate. In recent years, the incidence of craniocerebral trauma has been increasing with the development of transportation and construction industry. After injury, the combination of primary injury factors and secondary injury factors can cause different levels of nerve injury and leave behind the corresponding clinical symptoms[1,2]. Brain edema is one of the important pathological performances after craniocerebral injury, which is mainly characterized by increased water in brain tissue, can further elevate intracranial pressure and increase secondary brain damage, and can even cause cerebral hernia and be life-threatening in severe cases[3,4]. At present, the mechanism of cerebral edema after craniocerebral injury is not clear. The cytotoxic effect caused by pro-apoptosis molecules, oxygen free radicals, inflammatory factors and other toxic metabolites can destroy the integrity of the blood-brain barrier and cell structure, which increases water seepage and cause cerebral edema; the changes in aquaporins (AQPs) expression can affect the transport of water molecules, which results in water accumulation and causes brain edema. In the following studies, we took closed craniocerebral injury rat models as the research subjects, and specifically analyzed the relationship of cerebral edema with apoptosis, inflammatory factor generation and AQPs expression.
2. Experimental materials and methods

2.1 Experimental animals

A total of 30 SPF SD male rats purchased from the experimental animal center of Chongqing Medical University were selected as the experimental animals, animal license number was SCXK (Chongqing) 2007-0001, and the body mass was 200-300 g. Animal experiments passed through the ethical review of the hospital, and the animal experiments and treatment after death were conducted according to the procedures.

2.2 Experimental materials

Rat stereotaxic apparatus was purchased from Shanghai Medical Instrument Company, the kits for mRNA expression detection were bought in Takara Company, protein extraction reagent was purchased from Shanghai Beyotime Company, and the enzyme-linked immunosorbent assay kits were purchased from Shanghai Westang Biotechnology Company.

2.3 Experimental methods

2.3.1 Animal grouping and model establishing

SD rats were randomly divided into model group and control group, model group were established into closed craniocerebral injury models, and the methods were as follows: the rat was given intraperitoneal injection of chloral hydrate for anesthesia, then the head was fixed on the stereotaxic apparatus, an anterior median incision was made after routine disinfection to expose the skull, a bone window with diameter of 5 mm was made in the left rear area 4.5 mm from anterior fontanelle, and the striking tube was placed on the bone window and strike it with 1.8 standard atmospheric pressure. Control group were anesthetized with reference to the model group and the bone window was made in the same position without striking.

2.3.2 Detection of water content of brain tissue

7 d after model establishment, the rats were decapitated, the specimen collection was carried out on ice, the left side of the brain tissue was collected, meninges were removed, the moisture and blood were fully absorbed, about 30mg of brain tissue was cut from the damaged area to weigh the wet weight, then baked in 105 °C oven for 24 h and taken out to weigh the dry weight, and the brain tissue water content was calculated according to the formula (wet weight - dry weight)/wet weight. The residual brain tissue in the damaged area was used for the detection of mRNA expression and protein production.

2.3.3 Detection of gene mRNA expression in brain tissue

Appropriate amount of brain tissue was collected from the injured area, and the RNA extraction kit and reverse transcription kit were used to separate RNA from the brain tissue and synthesize it into cDNA by reverse transcription. Cdk5, Homer1a, Pim-3, Bcl-2, FasL, Caspase-3, AQP1, AQP4 and AQP9 primers were designed, fluorescence quantitative PCR kits were used to amplify cDNA, and the PCR reaction curve was referred to calculate Cdk5, Homer1a, Pim-3, Bcl-2, FasL, Caspase-3, AQP1, AQP4 and AQP9 mRNA expression.

2.3.4 Detection of protein generation in brain tissue

Appropriate amount of brain tissue was collected from the injured area, the total protein in the brain tissue was extracted by protein extraction reagent, and the generation of NF-kB, TNF-α, IL-1, IL-6 and p-JNK were determined by the enzyme linked immunosorbent assay kit.

2.4 Statistical methods

SPSS 20.0 software was used to input data, measurement data between two groups were by t test, and \( P < 0.05 \) meant statistical significance in differences in test results.

3. Results

3.1 Water content of brain tissue

The water content of damaged brain tissue of model group and control group were (86.41±10.93)% and (73.87±8.15)% respectively. Analysis of the water content of damaged brain tissue between two groups of rats was as follows: the water content of damaged brain tissue of model group was significantly higher than that of control group, and the differences in the water content of damaged brain tissue were statistically significant between two groups of rats \( (P<0.05) \).

3.2 Cdk5, Homer1a, Pim–3, Bcl–2, FasL and Caspase–3 expression in brain tissue

Analysis of Cdk5, Homer1a, Pim-3, Bcl-2, FasL and Caspase-3 expression in brain tissue between two groups of rats was as follows: Homer1a, Pim-3 and Bcl-2 mRNA expression in brain tissue of model group were greatly lower than those of control group whereas Cdk5, FasL, and Caspase-3 mRNA expression were greatly higher than those of control group. Pearson test showed that the water content of brain tissue of model group was negatively correlated with Homer1a, Pim-3 and Bcl-2 mRNA expression, and positively correlated with Cdk5, FasL and Caspase-3 mRNA expression.

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Cdk5</th>
<th>Homer1a</th>
<th>Pim-3</th>
<th>Bcl-2</th>
<th>FasL</th>
<th>Caspase-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model group</td>
<td>10</td>
<td>2.47±0.41</td>
<td>0.39±0.06</td>
<td>0.44±0.07</td>
<td>0.28±0.05</td>
<td>2.91±0.39</td>
<td>3.18±0.49</td>
</tr>
<tr>
<td>Control group</td>
<td>10</td>
<td>1.05±0.12</td>
<td>0.98±0.11</td>
<td>1.03±0.17</td>
<td>1.06±0.14</td>
<td>0.95±0.14</td>
<td>0.99±0.13</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

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Table 2.
Inflammatory factor generation in brain tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>NF-κB (ng/mL)</th>
<th>TNF-α (ng/mL)</th>
<th>IL-1β (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>p-JNK (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model group</td>
<td>10</td>
<td>4.25±0.67</td>
<td>22.45±3.49</td>
<td>7.59±0.93</td>
<td>10.39±1.35</td>
<td>0.82±0.11</td>
</tr>
<tr>
<td>Control group</td>
<td>10</td>
<td>1.84±0.24</td>
<td>9.48±1.38</td>
<td>3.14±0.45</td>
<td>4.29±0.58</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.3 NF-κB, TNF-α, IL-1β, IL-6 and p-JNK generation in brain tissue

Analysis of inflammatory factors NF-κB (ng/mL), TNF-α (pg/mL), IL-1β (ng/mL), IL-6 (pg/mL) and p-JNK (pg/mL) generation in brain tissue between two groups of rats was as follows: the above inflammatory factor generation in brain tissue of model group were greatly higher than those of control group. Pearson test showed that the water content of brain tissue of model group was positively correlated with NF-κB, TNF-α, IL-1β, IL-6 and p-JNK generation.

3.4 AQPs family molecule expression in brain tissue

Analysis of AQPs family molecules AQP1, AQP4 and AQP9 expression in brain tissue between two groups of rats was as follows: AQP1, AQP4 and AQP9 mRNA expression in brain tissue of model group were greatly lower than those of control group. Pearson test showed that the water content of brain tissue of model group was negatively correlated with AQP1, AQP4 and AQP9 mRNA expression.

Table 3.
AQPs family molecule expression in brain tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>AQP1</th>
<th>AQP4</th>
<th>AQP9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model group</td>
<td>10</td>
<td>0.36±0.06</td>
<td>0.28±0.05</td>
<td>0.31±0.07</td>
</tr>
<tr>
<td>Control group</td>
<td>10</td>
<td>1.04±0.17</td>
<td>1.01±0.15</td>
<td>0.97±0.14</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>19.395</td>
<td>25.685</td>
<td>21.382</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

4. Discussion

Cranioencebral injury can cause nerve damage by primary injury and secondary injury, and thereby result in higher fatality rate and disability rate. The mechanical damage to cranioencebral tissue caused by external violence is the primary injury factor, which can be removed in time through emergency surgery; secondary injury factor refers to the nerve injury caused by the pro-apoptosis molecules, oxygen free radicals, inflammatory factors and other toxic metabolites, and the damage factor persists after injury, causes adverse effects on the recovery of neurological function, and is a clinical hotspot of research[5,6]. Cerebral edema is an important pathological feature in the pathogenesis of cranioencebral injury, which is not only the outcome of primary injury, but can also become secondary injury factor to aggravate the degree of brain injury[7]. The characteristic of cerebral edema is the increase of water and volume of brain tissue, which can further increase intracranial pressure and cause the compression injury of brain tissue. Existing study shows that the pathophysiological mechanisms of cerebral edema include cytotoxic cerebral edema and angiogenic cerebral edema[8]. In the process of cranioencebral injury, the pro-apoptosis molecules, oxygen free radicals, inflammatory factors and other toxic metabolites can on the one hand, cause cytotoxic effect, and on the other hand, also destroy the blood-brain barrier and increase the permeability of water molecules, but the specific mechanism of cerebral edema is not clear. Analysis of the water content of brain tissue of closed cranioencebral injury model rats in the study showed that the water content of brain tissue of model group was significantly higher than that of control group. This indicates that the water content of brain tissue has increased in the process of closed cranioencebral injury, which also indicates that cerebral edema occurs in the process of closed cranioencebral injury.

Apoptosis is an important secondary pathological factor that causes nerve injury after cranioencebral injury, and the expression of various pro-apoptosis and anti-apoptosis molecules change significantly in this process. Homer1a is the Homer family member with anti-apoptotic activity, which can antagonize the toxicity of excitatory amino acids and calcium overload to inhibit apoptosis[9]; Pim-3 and Bcl-2 are the molecules that antagonize mitochondrial pathway of apoptosis, which can reduce the release of cytochrome C and the activation of its downstream apoptosis response[10]; Cdk5 is the Cdk family member with pro-apoptotic activity, which is activated under the action of excitatory amino acids, free radicals and other toxic substances, and then can be combined with various apoptotic substrates[11]; FasL is the regulatory molecule of apoptosis of the death receptor pathway, which can initiate apoptosis by FADD domain[12]; caspase-3 is the apoptosis executor that can cause DNA fracture in the nucleus, and different apoptotic pathways can ultimately induce apoptosis through the activation of caspase-3[13]. In the research, the analysis of above apoptosis molecule expression in the process of cranioencebral injury showed that Homer1a, Pim-3 and Bcl-2 mRNA expression in brain tissue of model group were greatly lower than those of control group whereas Cdk5, FasL and Caspase-3 mRNA expression were greatly higher than those of control group. This indicates that the expression of anti-apoptosis molecules decreases, the expression of pro-apoptosis molecules increases, and the apoptosis is aggravated significantly in the process of closed cranioencebral injury. Further analysis of the relationship between apoptosis and cerebral edema in the process of cranioencebral injury showed that the water content of brain tissue of model group was negatively correlated with Homer1a, Pim-3 and Bcl-2 mRNA expression, and positively correlated with Cdk5, FasL and Caspase-3 mRNA expression. This indicates that the increase of apoptosis in the process of cranioencebral injury is closely related to the occurrence of cerebral edema.

Inflammatory response is another secondary injury factor in the process of cranioencebral injury, and various inflammatory factors are massively secreted in the process. NF-κB is a transcription factor in cells that regulates inflammatory response, which can activate the transcription of multiple inflammatory cytokines after...
it is activated and enters the nucleus[14,15]. Inflammatory mediators TNF-$\alpha$, IL-1$\beta$ and IL-6 are all molecules regulated by NF-kB, and TNF-$\alpha$, IL-1$\beta$ and IL-6 that are massively secreted in the process of craniocerebral injury can on the one hand, cause inflammation cascade amplification and result in neuron injury and blood-brain barrier damage, and on the other hand, increase apoptosis by phosphorylation of the downstream JNK[16,17]. The analysis of the changes in the above inflammatory factor generation in the process of craniocerebral injury showed that the above inflammatory factor generation in brain tissue of model group were greatly higher than those of control group. This indicates that the inflammatory factors are excessively generated in the process of closed craniocerebral injury, and the inflammatory that are massively generated in local area can cause cell injury and blood-brain barrier damage. Further analysis of the correlation between inflammatory factors and cerebral edema in craniocerebral injury models showed that the water content of brain tissue of model group was positively correlated with NF-kB, TNF-$\alpha$, IL-1$\beta$, IL-6 and p-JNK generation. This indicates that the increase of inflammatory cytokines during craniocerebral injury is closely related to the occurrence of cerebral edema.

The water molecule distribution and transport are abnormal in the process of brain edema. The cytotoxic effect mediated by apoptosis and inflammation will influence the distribution of water molecules, and multiple members of the AQPs family can also affect the distribution and transport of water molecules[18]. AQP-1, AQP-4 and AQP-9 are three kinds of AQPs molecules expressed in the nervous system, which are widely distributed in glial cells, endothelial cells and epithelial cells, and can promote the water molecule transport and avoid abnormal water accumulation[19]. Analysis of the above AQPs family molecule expression in the process of craniocerebral injury in the study showed that AQP1, AQP4 and AQP9 mRNA expression in brain tissue of model group were greatly lower than those of control group. This indicates that the expression of many kinds of AQPs decrease in the process of closed craniocerebral injury, and the lowly expressed AQPs can affect the transport of water molecules and participate in the formation of local tissue edema. Further analysis of the correlation between AQPs expression and cerebral edema in craniocerebral injury models showed that the water content of brain tissue of model group was negatively correlated with AQP1, AQP4 and AQP9 mRNA expression. This indicates that the decreased expression of AQPs molecules in the process of craniocerebral injury is closely related to the occurrence of cerebral edema.

To sum up, it can be concluded that there is significant cerebral edema during closed craniocerebral injury; the excessive apoptosis, increased inflammatory cytokine generation and decreased AQPs expression are the important pathological factors for cerebral edema.

References