Evaluation of the blood coagulation function, degree of inflammation as well as apoptosis in brain tissue of rat models with heat stroke

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Abstract

Objective: To study the blood coagulation function, degree of inflammation as well as apoptosis in brain tissue of rat models with heat stroke. Methods: Healthy male SD rats were selected as experimental animals and randomly divided into heat stroke group and control group, heat stroke group were raised under the condition of environment temperature (40.0±0.5) °C and relative humidity 60% to establish heat stroke models, the anus temperature reached 42.5 °C, and the control group were conventionally raised; 60 and 120 min after model establishment, the blood coagulation function, serum inflammatory factor levels as well as brain water content, the number of apoptotic cells and apoptotic molecule expression were determined respectively. Results: (1) blood biochemical indexes: Activated partial thromboplastin time (APTT) and prothrombin time (PT) of heat stroke group were longer than those of control group, and serum D-Dimer content (D-D), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 levels were significantly higher than those of control group (P<0.05); (2) the brain tissue damage indexes: brain water content, the number of apoptotic cells, Caspase-3 expression and LC3-II/LC3-I ratio of heat stroke group were significantly higher than those of control group (P<0.05). Conclusions: There are blood coagulation dysfunction and systemic inflammation activation in heat stroke model rats, and the apoptosis in brain tissue increases obviously.

1. Introduction

Heatstroke is the disease that is characterized by the heat-regulating center dysfunction, sweat gland non-function and excessive water-electrolyte loss caused by high-temperature and high-humidity environment, including heat cramps, heat exhaustion and heat stroke[1,2]. Heat stroke is the most severe type of heatstroke, it is mainly characterized by core temperature exceeding 40 °C and association of consciousness disorder, and the clinical treatment is difficult and case fatality rate is high[3]. At present, studies on heat stroke believe that neurologic injury, multiple organ failure and disseminated intravascular coagulation are the common causes of deaths for patients with heat stroke[4,5], but the pathophysiological characteristics of heat stroke are still not completely clear. Blood coagulation dysfunction is the main characteristic of disseminated intravascular coagulation, inflammation is the main link mediating multiple organ failure, and the cell apoptosis within the brain tissue is closely related to the neurological damage. In the following study, the heat stroke model rats were established under high-temperature and high-humidity environment, and the blood coagulation function, degree of inflammation and apoptosis in brain tissue of model rats were analyzed.

2. Materials and methods

2.1. Experimental materials

Experimental animals were healthy male SD rats, they were bought in Beijing Vital River Laboratory Animal Technology Co., Ltd., and the animal license number was SCXK 2012009. Intelligent simulation lab was purchased from Tianjin Hope Company, fluorescence microscope was bought from the Nikon.
Company and protein visualizer was bought from Shanghai Tanon Company; TUNEL staining kits were bought from Roche Company, and Caspase-3 and LC3 monoclonal antibody were bought from CST Company.

2.2. Heat stroke model establishment methods

SD rats were randomly divided into heat stroke group and control group. The heat stroke group were placed in a specific environment-intelligent simulation cabin with the environment temperature maintained at (40.0±0.5) °C and relative humidity at 60%; after experimental rats entered the environment, the anus temperature was monitored once every 20 min, the anus temperature ≥42.5 °C indicated successful establishment of heat stroke models, and the rats with anal temperature reaching 42.5 °C continued to be placed in high-temperature and high-humidity environment, 6 rats were put to death after 60 and 120 min respectively, and the brain tissue and serum samples were collected for subsequent testing.

2.3. Blood biochemical index detection methods

60 and 120 min after model establishment, the rats were put to death, blood specimens were collected, coagulation analyzer was used to measure activated partial thromboplastin time (APTT) and prothrombin time (PT), fully automatic biochemical analyzer was used to determine D-Dimer content (D-D), and the enzyme-linked immunosorbent assay kits were used to determine the content of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6.

2.4. Brain water content detection methods

60 and 120 min after model establishment, the rats were put to death and anatomized to get whole brain tissue, the meninx was stripped, the blood was wiped, and the wet weight of the whole brain was weighed in electronic balance; then the whole brain tissue was place in 105 °C oven and baked to constant weight, and after that, the dry weight of the whole brain was weighed in electronic balance. The following formula was used to calculate the brain water content: (wet weight of whole brain - dry weight of whole brain)/ wet weight of whole brain.

2.5. TUNEL staining methods

Half of the dried whole brain tissue was collected, made into frozen sections, closed in the 3% hydrogen peroxide-methanol solution for 10 min, washed with PBS twice, permeated in 0.1% TritonX-100 solution for 2 min, then added in TUNEL reaction liquid for 1 h of incubation at 37 °C away from light, added in POD conversion liquid for 0.5 h of incubation at 37 °C away from light, finally added DAB for 10 min of development at room temperature, and counterstained with hematoxylin. 5 random high power fields were observed under fluorescence microscope, and the number of TUNEL positive staining cells was counted.

2.6. Apoptotic molecule expression detection methods

The other half of the dried whole brain tissue was collected, added in protein lysis buffer RIPA, fully homogenized and centrifuged to separate the tissue protein samples; Western-Blot kits were used to configure polyacrylamide gel and add it in the protein samples for vertical electrophoresis and horizontal transmembrane, the first antibodies of Caspase-3, LC3 and GAPDH were incubated respectively, the second antibodies were incubated after 24 h, development was conducted, and then the Caspase-3, LC3-I and LC3-II protein expression as well as LC3-II/LC3-I ratio were calculated.

2.7. Statistical analysis

Experimental data were input in statistical software package SPSS19.0, t test was used to analyze the differences in measurement data between two groups and P<0.05 indicated statistical significance in differences.

3. Results

3.1. Blood coagulation function indexes

60 and 120 min after model establishment, analysis of blood coagulation function indexes PT, APTT and D-D between two groups of rats is as follows: APTT and PT of heat stroke group were significantly longer than those of control group, and serum D-D level was significantly higher than that of control group (P<0.05) (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>60 min after model establishment</th>
<th>120 min after model establishment</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>APTT (s)</td>
<td>PT (s)</td>
</tr>
<tr>
<td>Heat stroke group</td>
<td>58.76±7.82</td>
<td>27.84±3.35</td>
</tr>
<tr>
<td>Control group</td>
<td>43.58±6.15</td>
<td>15.79±1.83</td>
</tr>
<tr>
<td>t</td>
<td>6.789</td>
<td>7.481</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
3.2. Inflammation indexes

60 and 120 min after model establishment, analysis of serum inflammation indexes TNF-α, IL-1β and IL-6 levels between two groups of rats is as follows: serum TNF-α, IL-1β and IL-6 levels of heat stroke group were significantly higher than those of control group ($P<0.05$) (Table 2).

3.3. Brain injury indexes

60 and 120 min after model establishment, analysis of brain water content, number of apoptotic cells and apoptotic molecule expression in brain tissue between two groups of rats is shown in Table 3: brain water content and the number of apoptotic cells of heat stroke group were significantly higher than those of control group; Caspase-3 expression and LC3-II/LC3-I ratio in brain tissue of heat stroke group were significantly higher than those of control group ($P<0.05$).

4. Discussion

Heat stroke is the most severe type of heatstroke, which is with high case fatality rate and difficult clinical treatment[6]. Nerve injury, multiple organ failure and disseminated intravascular coagulation caused by temperature rising are the common causes of deaths for patients with heat stroke[7,8], but the pathophysiology of above causes of death has not been fully elucidated. Blood coagulation dysfunction is an important pathological change in the occurrence and development of disseminated intravascular coagulation, and constantly consumption of blood coagulation factors can cause intravascular coagulation as well as secondary coagulation dysfunction and fibrinolytic hyperthyroidism[9]. In the study, comparison of blood coagulation function indexes between heat stroke model rats and normal rats showed that PT and APTT of heat stroke group were significantly longer than those of control group, and serum D-D level was significantly higher than that of control group ($P<0.05$). It is believed that: PT and APTT are the indicators that reflect endogenous blood coagulation function and exogenous coagulation function respectively, and the significant extension of PT and APTT can indicate that there are significant endogenous and exogenous coagulation dysfunction in heat stroke model rats at the same time; D-D is the specific degradation product after fibrin monomer crosslinks and then is hydrolyzed by fibrinolytic enzyme, it can reflect the dissolution function of fibrin, and the increase in serum D-D levels indicates that there is secondary fibrinolytic hyperfunction in heat stroke model rats.

In the development and change of heat stroke, systemic inflammatory response activation and massive inflammatory medium release are the important mechanisms of the blood coagulation dysfunction, and the systemic inflammatory response syndrome mediated by inflammatory media can also cause multiple viscera function damage and increase the occurrence risk of multiple organ dysfunction syndrome. TNF-α, IL-1β and IL-6 are the important media mediating systemic inflammatory response syndrome[10]. IL-1β and IL-6 are the cytokines that first change in the process of inflammation caused by heat stroke, and they are closely related to multiple viscera function damage and regulation of other inflammatory factor secretion; TNF-α is the cytokine with both proinflammatory and inflammatory tissue damage effect, and it can not only activate the cascade of the inflammatory cytokines and amplify inflammation, but can also cause tissue damage and viscera function failure[11,12]. In the study, comparison of serum inflammatory factors between heat stroke model rats and normal rats showed that serum TNF-α, IL-1β and IL-6 levels of heat stroke group were significantly higher than those of control group.

### Table 2
Comparison of serum inflammation indexes between two groups of rats (ng/mL, $n=5$, $\overline{\text{x}} \pm s$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>60 min after model establishment</th>
<th>120 min after model establishment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF-α</td>
<td>IL-1β</td>
</tr>
<tr>
<td>Heat stroke</td>
<td>37.68±5.14</td>
<td>17.49±2.25</td>
</tr>
<tr>
<td>Control group</td>
<td>20.35±3.29</td>
<td>10.38±1.27</td>
</tr>
<tr>
<td>$t$</td>
<td>8.697</td>
<td>8.175</td>
</tr>
<tr>
<td>$P$</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.05$</td>
</tr>
</tbody>
</table>

### Table 3
Comparison of brain water content, number of apoptotic cells and apoptotic molecule expression in brain tissue between two groups of rats ($n=5$, $\overline{\text{x}} \pm s$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>60 min after model establishment</th>
<th>120 min after model establishment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water content</td>
<td>Number of apoptotic cells</td>
</tr>
<tr>
<td>Heat stroke</td>
<td>77.61±8.69</td>
<td>16.85±2.16</td>
</tr>
<tr>
<td>Control group</td>
<td>68.76±8.54</td>
<td>8.57±0.92</td>
</tr>
<tr>
<td>$t$</td>
<td>7.617</td>
<td>9.689</td>
</tr>
<tr>
<td>$P$</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.05$</td>
</tr>
</tbody>
</table>
(P<0.05). This means that in the development and change of heat stroke, the release of inflammatory mediators TNF-α, IL-1β and IL-6 significantly increases, which can activate the systemic inflammatory response to cause tissue structure damage and viscera function failure.

Systemic inflammatory response syndrome caused by hyperpyrexia can greatly increase the risk of multiple organ dysfunction syndromes, the brain is the most common involved target organ in the development of heat stroke, continuous high temperature will directly cause nerve function damage, and the constantly generated inflammatory cytokines can also cause secondary inflammatory brain injury. The most prominent histologic manifestations of nerve injury caused by heat stroke are the aggravated brain edema and the increased number of apoptotic cells between heat stroke model rats and normal rats in the study showed that brain water content and the number of apoptotic cells of heat stroke group were significantly higher than those of control group (P<0.05). This shows that there are significant cerebral edema and cell apoptosis in rats with heat stroke. The Caspase-3-mediated apoptotic signaling pathway and the LC3-mediated autophagy pathway are the important mechanisms of apoptosis in brain tissue[14,15]. Caspase-3 is regulated by upstream mitochondrial apoptosis pathway and death receptor apoptosis pathway, and it activates and directly mediates cell apoptosis process through the cascade amplification-mediated by Caspase family[16]; LC3 is a marker molecule of cell autophagy, and in the process of autophagy activation, LC3-I transition to LC3-II ratio also increases correspondingly[17]. This means that the excessive apoptosis in brain tissue of heat stroke rats is mediated by Caspase-3 apoptosis pathways and LC3 autophagy pathway.

To sum up, the pathophysiological characteristics of heat stroke model rats include the secondary coagulation dysfunction and fibrinolytic hyperthyroidism, systemic inflammatory response activation as well as significantly increased cell apoptosis and autophagy in brain tissue.

References


