Changes and Significance of Cytokines in Serum of Rabbits with Acute Pulmonary Embolism

Yulong Liang* Jianjing Liang2 Wenjing Feng1 Jinghui Mu1
1. General Surgery, Third Hospital of Hebei Medical University, Shijiazhuang, Hebei, 050000, China
2. Hebei University, Baoding, Hebei, 071000, China

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ABSTRACT

Objective: To observe the changes of cytokines in serum of rabbits with acute pulmonary embolism, and to provide a scientific basis for clinical treatment of the disease.

Methods: The animal models of pulmonary embolism were established in 26 healthy rabbits by autologous thrombus reinfusion and normal saline injection, and the serum levels of cytokines (TNF-α, IL-1β, HGF) in the two groups were monitored by enzyme-linked immunosorbent assay.

Results: The expression levels of serum inflammatory cytokines (TNF-α, IL-1β) increased in both groups of rabbits within 1-3 hours of the acute stage of embolism, but the expression levels of serum inflammatory cytokines in experimental group of rabbits with pulmonary embolism caused by thrombosis increased more obviously. There was no significant change in serum HGF of rabbits before and after embolization in group B. The serum HGF content of experimental rabbits at 1 h, 3 h, 12 h, 24 h and 48 h after embolization was significantly higher than that before embolization, and in the acute stage of embolism (1-12 hours), it showed a gradual upward trend.

Conclusions: The expression level of serum growth factor is different in different acute stages of pulmonary embolism. Detecting serum cytokines in rabbits with acute pulmonary embolism is of great reference significance for improving clinical diagnosis.

1. Introduction

Pulmonary thromboembolism (PTE) refers to a disease in which pulmonary artery is blocked by detached thrombus or other substances, which belongs to a branch of peripheral vascular diseases. Typical symptoms include dyspnea, chest pain, hemoptysis, fever, etc. After embolization, the blood supply function of pulmonary artery in patients will decrease, which will cause lung tissue necrosis and induce pulmonary infarction in a short time, leading to pulmonary dysfunction in patients, with extremely high disability mortality rate, and seriously threatening human health and life safety. PTE has no special clinical symptoms, and there is no specific diagnosis method for this disease at present. Therefore, at first, the medical community in China has formed a consensus on this disease,
that is, it is a rare disease in China, which also leads to a high misdiagnosis rate and missed diagnosis rate of this disease clinically in China. However, with the continuous development and progress of medical technology, through the unremitting exploration of countless clinical medical scholars, the research on this disease in China’s medical field has made substantial progress. In recent years, the efficiency of finding and diagnosing this disease in bed has become higher and higher, which provides a favorable direction for the next clinical treatment. Studies have shown that the expression level of serum cell growth factor has obvious changes in the early stage of pulmonary dysfunction, suggesting that the expression level of serum cell growth factor has guiding value for the early diagnosis of acute thrombotic pulmonary embolism. In view of this, in this study, rabbits were selected as the research object to establish an animal model of pulmonary embolism by autologous thrombus reinfusion, and the changes of growth factors in serum of rabbits with acute thrombotic pulmonary embolism were observed, so as to provide scientific basis for further clinical study of the pathological process of acute pulmonary embolism and more effective clinical treatment.[1].

2. Data and Methods

Twenty-six healthy pure New Zealand white rabbits were selected as the research object, and they were divided into control group and experimental group according to the random principle, with 13 rabbits in each group. In the second group, there were 5 female rabbits and 8 male rabbits, the maximum body weight was 3.8 kg, the minimum body weight was 2.9 kg, the average age was (3.24±0.32) kg, and the average age was (5.2±0.8) months. In the experiment group, there were 6 female rabbits and 7 male rabbits, with the largest body weight of 3.6 kg, the smallest of 3 kg, and the average age of (3.31±0.26) kg, 4-7 months, and the average age of (5.3±0.8) months. The data of the two groups of rabbits showed that the initial state was similar, and the difference of general conditions between the two groups was not significant.

2.1 Research Materials

Iodophor disinfectant, intravenous syringe, sterile catheter, medical water bath box, normal saline, phenobarbital sodium anesthetic, 5F sheath dilator, suture device, centrifuge, enzyme-linked immune costume experimental kit (produced by Jinan Kesheng Medical Technology) [2].

2.2 Method

Before the start of the experiment, a tandem immuno-adsorption method was used. The experimental rabbits in group B adopted autologous thrombus reinfusion method to establish pulmonary embolism model. The first step is to prepare embolus. Select the rabbit ear vein to extract appropriate amount of blood and inject it into sterile catheter. In order to ensure the accuracy of blood test results, the rabbit ear vein should be disinfected locally before blood extraction. Set the catheter at rest until the blood is coagulated, then put it in the water bath box, adjust the temperature of the water bath box to 70 °C for 10 minutes, then use disposable syringe to push the thrombus in the catheter into the sterile bending plate, and make it into a 3-4 mm embolus, and rinse it repeatedly with normal saline for later use. The second step is autologous thrombus reinfusion. The rabbits were anesthetized by intraperitoneal injection of phenobarbital sodium at a dose of 150 mg/kg. Then, the rabbits were fixed, and femoral venous autologous thrombus was selected for reinfusion. Firstly, after removing the leaked skin from the thigh rabbit hair, routine disinfection was performed with iodophor, and then Seldinger puncture was performed along the femoral artery. The 5F sheath expansion tube with an inner diameter of 0.056 in inch produced by Cordis Company was selected and inserted into the right atrium. The emboli prepared in advance by the sheath expansion tube were injected in several times. In order to ensure the successful reinfusion of the emboli and avoid their staying in the sheath expansion tube, after each injection of embolus, inject 2 mL of normal saline into the expanded tube of the sheath for flushing. Repeat the injection of embolus to ensure that the amount of embolus reinfusion is more than 1 mL/kg, or stop the injection when the rabbits have obvious symptoms of hypoxia such as shortness of breath and cyanosis of the lips. In the same way, the rabbits in the control group were injected with normal saline. Combined with the results of pulmonary angiography and pathological section, pulmonary embolism in this group of rabbits was confirmed [3].

2.3 Observation Indicators

After successful modeling, 1 mL of rabbit ear vein blood was taken and centrifuged at 300 r/min. The supernatant was extracted and the expression levels of tumor necrosis factor-α (TNF-α), inflammatory cytokine interleukin-1β (IL-1β) and hepatocyte growth factor (HGF) in serum of two groups of rabbits were measured by enzyme-linked immunosorbent assay at 1 h, 3 h, 12 h, 24 h and 48 h after embolization. After the experiment, rabbits were killed by injecting excessive anesthetic, dissected, and lung tissue was taken to observe the distribution and mor-
phology of emboli in pulmonary vessels. A small amount of pulmonary embolism tissues were embedded in graphite, then treated with HE staining, and pathological observation was made with the aid of medical microscope.  

2.4 Data Processing

Using SPSS20.0 statistical software, the data of counting data (n, %) and measuring data (s) were processed by chi-square test and t test, with P<0.05 being statistically significant.

3. Results

3.1 Expression Level of Inflammatory Cytokines in Serum

According to the data in Table 1, before pulmonary embolism, the expression levels of inflammatory cy-

tokines (TNF-α, IL-1β) in serum of two groups of rabbits were similar, and after pulmonary embolism, the expression levels of inflammatory cytokines in serum of two groups of rabbits were different at different times. The expression level of inflammatory cytokines in serum of rabbits in both groups increased within 1-3 hours of acute embolism, but the expression level of inflammatory cytokines in serum of rabbits in experimental group with pulmonary embolism caused by thrombosis increased more obviously, as shown in Table 1.

3.2 Results of Pathological Examination of Lung Tissue

Compared with the control group, the pathological examination of rabbit lung tissue in the experimental group showed that there were many dark red bleeding spots on the lung surface, many thrombi in the pulmonary artery and severe alveolar atrophy.

Table 1. Comparison of serum inflammatory cytokines expression levels between two groups (±s)

<table>
<thead>
<tr>
<th>Serum index</th>
<th>group</th>
<th>Before embolization</th>
<th>Post embolism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>TNF-α (ng/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control group</td>
<td>2.06±0.61</td>
<td>2.07±0.63</td>
<td>2.11±0.67</td>
</tr>
<tr>
<td>Experimental group</td>
<td>2.11±0.72</td>
<td>3.43±0.76</td>
<td>5.19±0.83</td>
</tr>
<tr>
<td>IL-1β (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control group</td>
<td>85.03±7.12</td>
<td>85.26±7.06</td>
<td>94.27±8.34</td>
</tr>
<tr>
<td>Experimental group</td>
<td>86.15±8.71</td>
<td>119.27±11.34</td>
<td>183.01±30.25</td>
</tr>
</tbody>
</table>

4. Discussion

Acute pulmonary embolism is an extremely dangerous disease, which can be divided into thrombotic pulmonary embolism, fat embolism, amniotic fluid embolism and air embolism according to the pathogenic factors, among which thrombotic pulmonary embolism is the most common one. The cause of the disease is complicated, and there is no conclusion in the medical field. However, according to relevant literature, many inflammatory mediators of cytokine network may play a certain role in the formation of the disease. In addition, some studies have found that after acute lung injury, the expression of hepatocyte growth factor in serum will be abnormal. Then, as a type of lung injury, will the serum hepatocyte content change? In order to test this conjecture, combined with the theory that inflammatory mediators participate in pathology, this study selected 26 rabbits for grouping study, of which 13 rabbits established acute pulmonary embolism model by thrombus reinfusion, and the other 13 rabbits were injected with normal saline in the same way, and then the expression levels of inflammatory cytokines and hepatocyte factors in serum of two groups of rabbits were measured by enzyme-linked immunosorbent assay. The results showed that before pulmonary embolism, the expression levels of inflammatory cytokines (TNF-α, IL-1β) and hepatocyte factor (HGF) in serum of two groups of rabbits were similar. After the occurrence of pulmonary embolism, the serum inflammatory cytokines of the two groups of rabbits have different expression levels at different times. The expression level of inflammatory cytokines in serum of rabbits in both groups increased within 1-3 hours of acute stage of embolism, but the expression level of inflammatory cytokines in serum of rabbits in experimental group with pulmonary embolism caused by thrombosis increased more obviously. The content of hepatocyte (HGF) in serum of rabbits in control group had no significant change before and after embolization. The expression level of HGF in serum of experimental rabbits in group A was significantly higher than that before embolization from 1 hour to 48 hours after embolization, and showed a continuous rising pattern.
within 1 to 1-12h hours. It shows that the expression of inflammatory cytokines and hepatocyte factors in serum of rabbits with thrombotic pulmonary embolism will be abnormally increased in acute.

To sum up, the changes of serum growth factors (TNF-α, IL-1β, HGF) in the rabbit model of acute pulmonary embolism can prove that the expression levels of serum growth factors in patients with different types of pulmonary embolism are different at different times, and the clinical reference value is accurate, which has far-reaching significance for accurate clinical diagnosis and symptomatic treatment.

References