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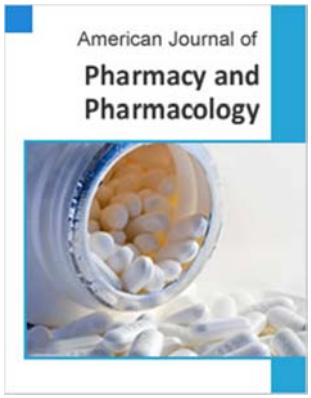

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Changes of Serum Procalcitonin in Rats with Cerebral Ischemia Reperfusion

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Abstract
In order to study the content of procalcitonin (PCT) in rat model of stroke reperfusion, and explore the feasibility of PCT as a sensitive marker of stroke, model group and sham operation group were set. A rat model of focal cerebral ischemia was established by modified suture method, then pulled out a threaded portion after MCAO reperfusion 1 hour. And then to the rats to take blood abdomen, finally with ELISA method to detect PCT 24 h and 48 h serum PCT level in rats. The results showed that the level of serum PCT in model group was significantly higher than that in sham operation group, which confirmed that the rat serum PCT can be used as a marker of stroke one of the markers used to detect the symptoms of ischemic stroke in rats.

1. Introduction

Stroke is a variety of reasons lead to a group of diseases cerebral vascular damage. Brain damage is the pathological basis of clinical symptom [1]. It is one of the important factors threatening human health. With high morbidity, disability and mortality characteristics, it is a serious threat to people's health [2]. Suffering from acute ischemic stroke patients of the most common clinical manifestations were fever, but the traditional detection method can not timely to stroke infection and fever causes were determined which leads to the abuse of antibiotics. Some researchers have found that PCT and stroke were closely related, not only can be used to identify 0arly indicators of [3] in stroke patients with fever, and can predict the occurrence of stroke associated pneumonia (stroke-associated pneumonia, SAP) [4]. Therefore exploring PCT that associated with stroke is very important. With the development of immunology and the progress of medical and health care, people's understanding for stroke is more and more comprehensive. This experiment selected application of procalcitonin infection in stroke in this direction, Experiment detect the serum level of PCT to diagnosis stroke.

2. Experimental Section

2.1. Experimental Materials

2.1.1. Experimental Animals

Healthy male SD rats 24, weight of 250 + 20g, SPF level, provided by the experimental animal center of Military Medical Science Academy of the PLA. License number: SCXK (Army) 2012-0004, feed SCXK (Beijing) 2015-0007.

2.1.2. Main Experimental Reagents and Instruments

The main experimental reagents: rat procalcitonin (PCT) ELISA Kit (Shanghai Kexing Biotechnology Co. Ltd.); 2,3,5 chloride three phenyl tetrazole (TTC) from Beijing Boao Extension Technology Co. Ltd., with the electronic scales weigh accurately 1g, add 100ml 0.1M, PH= 7.2-7.4PBS phosphate buffer water bath heating that is, mixing, dissolution related reagents; PBS phosphate (Beijing LEYBOLD Cable Technology Co. Ltd.); chloraldurat provided by Tianjin Kermel Chemical Reagent Co., accurately weighed 10g, added to 100mL normal saline water stir to dissolve, and save it to the reagent bottle made of chloral hydrate reagent.; paraformaldehyde provided by Tianjin Comio Chemical Reagent Co. Ltd., with the phosphate buffer solution, mixed reagent to 4% concentration.

The main experimental instruments: electronic balance (Ohaus Instruments Co. Ltd.); double beam UV visible spectrophotometer (TU-1901) (Beijing Purkinje General Instrument Co., Ltd); polyscience (DR-HW-2) (Beijing medical equipment factory); high speed refrigerated centrifuge (TG16W) (Changsha Yida Instrument Co. Ltd.); full automatic microplate reader (ELx800) (US BIO-TEK Baote).

2.2. Experimental Methods

24 SD rats, male, at 24-26 DEG C at room temperature in laboratory routine feeding 3 D, free feeding and drinking water. Rats of preoperative fasting 12h, free drinking water, weighing, were randomly divided into 4 groups as follows: in the model group, 12 hours group (M - 12), the model group 48 hour group (M - 48), the sham operation group 12 hours (J - 12), the sham operation group 48 hours (J - 48). Groups of rats after adaptive feeding after 3 days of formal experiments. Using the modified suture line preparation of cerebral ischemia model in rats. The first with 10% mass fraction of chloral hydrate 0.3mL /100g Web Cavity injection anesthesia, fixed neck skin preparation, disinfection, incision, blunt dissection of the neck muscles, isolated carotid artery (CCA), external carotid artery (ECA), internal carotid artery (ICA) ligation, ECA distal and proximal end of CCA artery. Clipping ICA, insert the MCAO thread by CCA the internal carotid artery (ICA), to promote, to the middle cerebral artery (MCA), leading to focal cerebral ischemia. 10-0 suture neck incision. Sham operation group animal insert MCAO suture, intraoperative strict control of room temperature in 20-25 C. 1H cerebral ischemia after thrombus Pull out the line, as the starting time of reperfusion. The thread can not pull out all should remain part of the common carotid artery, avoid the pull-out suture caused death hemorrhage in rats. Preoperative body weight of rats in control of 250g within the range of 280g, can increase the success rate.

After the rats with 10% chloral hydrate 1mL/100g anesthesia, abdominal aortic blood 5ml, blood was centrifuged at 3000r/min 10min, the supernatant serum -20 C refrigerator to be detected; from rat brain, cerebellum, brainstem, hydrangea, removed, weighed and frozen in the refrigerator -20 C 30min, crown shaped along the direction of the brain uniform cut into five pieces, water bath at 1% TTC and light 15min.

2.3. Index detection

Main outcome measures: body weight change, brain index, serum procalcitonin level, pathological score of lung tissue and neurological function score before and after the model

(1) Brain index:

Organ index = organ wet weight (mg) / body weight (g) * 100%

(2) Rat neurological impairment score

The degree of neurological impairment was evaluated by modified Zea Longa standard. The degree of neurological deficit was divided into six grades

Table 1. *Specific scoring criteria.*

6 evaluation criteria of nerve injury in rats	
0 points, no defects	3 points, slightly to the side.
1 minutes, unable to extend the contralateral forelimb	4, serious to the side.
2 minutes, contralateral forelimb bending	5 points, contralateral paralysis

(3) Detection of PCT. by ELISA

2.4. Statistical Methods

The experimental results are expressed as mean + standard deviation ($\bar{x}\pm s$). SPSS 19.0 software was used to analyze the differences between the groups. The statistical analysis was performed by t test, and the difference was statistically significant with $P<0.05$.

3. Experimental Results

3.1. General Situation of Rats

From the experimental data, the weight of all rats were reduced and 48h was less than 24h body weight. J-24, J-48 two rats in group 48h were no neurological damage, and the operation group rats had mild or severe contralateral circling, and along with the extension of time and present serious circling trend.

3.2. Brain Index of Rats in Each Group

Organ index = organ wet weight (mg) / body weight (g) * 100%

Table 2. *Comparison of brain index between sham operation group and model group (x±s).*

group	N	Brain index
J-48	6	0.56±0.14
J-24	6	0.56±0.11
M-48	6	0.52±0.10
M-24	6	0.53±0.14

3.3. Sham Operation Group and Model Group Brain Slices Image

J-24 slice J-48 slice M-24 slice M-48 slice

Figure 1. *The brain slices of cerebral ischemia rat model.*

The experimental rat brain were stained, the white part of the figure for the ischemic area, red blood supply area. The image can more directly reflect the situation of brain ischemia rat model. From the picture can be seen in the blood supply of the rats in the sham operation group is not affected, and did not cause cerebral ischemia.

3.4. Neurological Injury Score

Table 3. *Comparison of neurological function score between sham operation group and model group (x±s).*

group	N	Neurological function score
J-48	6	0
J-24	6	0
M-48	6	3.33±0.52
M-24	6	3.50±0.55

Analysis of score of neurological function in rats: Neurological function score of sham injury compared with the model group with significant difference in 24 hours ($p=0.0133<0.05$), Neurological function score of sham operation compared model has significant difference in 48 hours ($p=0.0133<0.05$). The other groups had no significant difference. The neurological scores of rats in model group was between 3-4 model making success.

3.5. Effect of on Serum Procalcitonin Level in Rats

Table 4. *Comparison of PCT content between sham operation group and model group (x±s).*

group	n	PCT content
J-48	6	215.92±70.28
J-24	6	320.33±89.27
M-48	6	350.78±62.31
M-24	6	493.98±95.16

For this experiment, we need to compare the four groups, J-24, J-48, J-24, M-24, M-24, M-48, J-48, M-48.

Analysis shows that J-24 from above, J-48 group $p=0.041<0.05$, M-24, M-48, J-24, $p=0.005<0.05$ group, M-24 group $p<0.05$, J-48, M-48 in group $p<0.05$ were significantly different. That model group rats serum PCT levels were significantly higher than those in sham operated rats serum PCT levels, the difference was statistically significant PCT. The level of rat serum in rats can be used to detect whether symptoms of ischemic stroke.

4. Conclusion

The experimental results showed that sham brain coefficient lower than that of model group, and there were no neurological damage in vitro indications, more brain sections showing the results of mechanical damage, while the model group and vice versa. Result prove the stroke rat model experiment is successful and has practical significance.

The experimental data proved: J-24, J-48 group $p=0.041<0.05$, M-24, M-48 group, $p=0.005<0.05$, J-24, M-24 $p<0.05$ group, J-48 group, M-48 $p<0.05$ were significantly different. That model group rats serum PCT levels were significantly higher than those in sham operated rats serum PCT levels, the difference was statistically significant, which indicat the level of PCT in serum of rats can be used for the detection of rats with ischemic stroke.

5. Discussion

Cerebrovascular disease is the first killer of disability and death in the city or in the country, 70% the reason for this phenomenon is which is one of the risk factors [5]. One of the risk factors is the influence of inflammation [6]. More older people suffer ischemic stroke, which with severe hemiplegia or paralysis symptoms and bring heavy the burden to family and society. The cerebral ischemia reperfusion injury (CIRI) of physiological and pathological processes involving the complex interactions between factors still need further study. CIRI mainly associated with the formation of free radicals is excessive, the toxicity of excitatory amino acids, calcium overload, inflammatory reaction. This disease cannot be treated quickly, and need to stay in bed for a long time for receiving treatment. At the same time because of the immunity of the patients were prone to damage the infectious disease, aggravating illness, increased mortality in patients with [7]. Diagnosis biological markers of traditional infectious diseases such as CRP, leukocyte count, erythrocyte sedimentation rate so, but its specificity is not strong and limited clinical value of pathogenic [8]. Although bacteria culture is the gold standard for diagnosis of infectious diseases, but it cost for the inspection of long time and has low positive rate, which brings certain difficulty for clinical diagnosis of stroke patients.

PCT is a glycoprotein molecules composed of 116 amino acids,which was close to 13kd. Under normal circumstances, PCT is produced by the thyroid C cells, serum half-life is 25 ~ 30h, the blood level of PCT in normal adults is not higher than 0.1ng/ml, almost could not be detected in [9]. In state of bacteria infection, plasma PCT the concentration increased rapidly, wihch can be detected in 2h and 6h increased sharply and maintain high levels in 8h to 24h [10]. For virus infection stimulated macrophages to synthesis gamma interferon

inhibits synthesis related factors of PCT, so the virus infection patients that content of PCT kept low level. So PCT is clinical index which considered to be effective in differential diagnosis of bacterial infection and non bacterial infections, and the diagnosis accuracy is better than body temperature, leukocyte count, erythrocyte sedimentation rate CRP and inflammatory factors such as [11].

In order to verify whether PCT can be used as a sensitive marker of biological stroke, we first construct the model of rat cerebral infarction. By constructing the model group and the sham operation group of two groups, and respective with brain coefficient, neurological injury score, weight change, body appearance and brain slice observation to observe and compare for validation if the rat model of the model group stroke is constructed successfully. Experiments show that the constructed model is successful and meaningful.

Single factor analysis of PCT content in following experiment: because the half-life of PCT is 25 ~ 30h, if we can achieve the implementation of dynamic detection of PCT levels which can better get clinical effect that PCT predict ischemic stroke. To do this, but this experiment through rat abdominal aorta blood test, so temporarily unable to achieve dynamic monitoring, only selected two time gradient, 24 hours and 48 hours. The results proved that serum levels of PCT in rat serum can be used to detect whether a rat is suffering from ischemic stroke.

However, in this experiment is established in stroke rat model without infection, and contact with J-24, M-48 group $P>0.05$, M-24, M-48 group $P<0.05$ data. It can explain that PCT content increased in rat mode of uninfected stroke in 24h, and decreased to normal level after 48h. To a certain extent can predict that the effect of PCT had interval property and PCT increased may be due to inflammatory factors. Early detection is not better, and no exact diagnosis may lead to misdiagnosis. Combined with the study of [12] Wu Dongchuan and other scholars, PCT level can not be obtained the difference between the infection and non infection in 24h, all showed an upward trend, and there will be a different trend in 24-48h. that is PCT content what increase in 24h-48h indicates infection, decline or return to normal indicates no infection.

Because clinical trials need more conditions to prepare, so we will be in the future based on the experimental data of uninfected rats to study the PCT content of the clinical stroke in 48h.

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