Traumatic brain injury research and expression of caveolin-1 and its relationship with disease prognosis

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Abstract: The objective of this paper is to study the expression of caveolin-1 in the traumatic brain injury patients and its relationship with disease prognosis. Caveolin-1 was measured in 52 patients with ventricular hemorrhage within 8h, 24h, 48h, 72h and 1 week after onset by enzyme-linked immunosorbent assay (ELISA), to observe the changes of cerebrospinal fluid caveolin-1. The level of caveolin-1 in the brain of all patients was higher than that of the control group at 8 h, 24h, 48 h, 72h and 1 weeks after the onset (P<0.05) and the level of caveolin-1 in cerebrospinal fluid (CSF) of the severe group was higher than that of the light-medium group within 8h, 24h, 48 h and 72h after the onset (P<0.05). The level of caveolin-1 in CSF was significantly increased in patients with ventricular hemorrhage within 8h, 24h, 48h, 72h and 1 weeks after onset, and the expression of caveolin-1 in brain was related to the severity of craniocerebral injury. Therefore, the expression of caveolin-1 can be used as an indicator of the prognosis of traumatic brain injury disease.

Keywords: Brain injury, caveolin-1, expression, prognosis.

INTRODUCTION

With the continuous increase in traffic accidents, construction accidents and violent crime, traumatic brain injury (TBI) has become a more common acute trauma, but also belongs to relatively harmful disease in traumatology. According to the relevant information, the incidence of traumatic brain injury accounts for the second rate of the total trauma rate, but its disability rate is in the first place (Wang, 2015). It is reported that up to 150 thousand people die of trauma each year in the USA, among which more than half of them are caused by brain injury. Meanwhile, the number of people dying from brain injury in Britain has accounted for 2/3 of the total number of death due to trauma (Nyquist *et al.*, 2007). Moreover, related studies have shown that the secondary injury and repair mechanism after brain injury is very complicated. And because of the bad micro-environment of the injury part, it is very difficult to regenerate the axon of mature central nervous system (CNS) (Gu *et al.*, 2012). Until now, relevant experts have yet to develop an effective way to effectively prevent various long-term complications caused by TBI (Fernandez-Hernando *et al.*, 2009). Therefore, how to reduce the disability rate of TBI has become one of the urgent problems that need to be solved in the field of medical today.

Existing in cytoplasmic membrane, the structure of Caveolae belongs to submicroscopic structure, with the diameter of about 50-100 nm. It enriches in various kinds of protein molecule such as sphingomyelin, glycosphingolipids and cholesterol. Caveolae is involved in a variety of pathophysiological process. For example, endocytosis and intercellular transport, the differentiation, proliferation, death and migration of cell, remodeling the extracellular environment, cell signal transduction and so on (Chang *et al.*, 2011). Caveolin-1 is not only the marker protein of Caveolae, but also the main regulator of signal transduction, which can regulate the function of them through the direct contact between the scaffold and the signal molecule, such as eNOS. And for some signals, protein can combine with its receptor to regulate signal transduction (Deng and Li, 2013). Caveolin-1 shows different changes in different pathophysiological processes, such as the increase or decrease of Caveolin-1 and the morphological changes in Caveolae (Huang *et al.*, 2015).

At present, the change of caveolin-1 in patients with brain injury has not been reported in China. In order to reveal its role in the pathogenesis process of cerebral hemorrhage, 52 cases received operation of craniocerebral injury in our hospital from February 2014 to October 2015 were selected to observe the change of caveolin-1 of patients with ventricular hemorrhage, and to explore the relationship between the severity of the disease and the change in the level of caveolin-1.

MATERIALS AND METHODS

General data

52 cases (male 24 cases, female 28 cases) were divided into two groups according to the GCS score on submission, namely, light-medium group (GCS score: 9-15) and severe group (GCS score: 3-8). There were 29 cases in light-medium group, aged between 43-62 years

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old, with an average age of 51 years old; there were 23 cases in severe group, aged between 39-58 years old, with an average age of 50 years old. The cause of all patients were suddenly headache or vomiting, and they were admitted to hospital 2-5 h after onset. Among them, 45 cases had a history of high blood pressure. Preoperative CT examination had confirmed the hematocele, expansion and casting of the ventricular system. 37 cases were primary intraventricular hemorrhage, and another 15 cases secondary ventricuiar hemorrhage. 8 cases were whole brain cast hemorrhage, and 32 cases bilateral lateral ventricle brain cast hemorrhage; 12 cases were oneside lateral ventricle brain cast hemorrhage and small amount of accumulated blood in the lateral ventricles. Among them 9 cases had obvious accumulation of blood in the third ventricle and the four ventricle.

The control group had 20 healthy people (male 10 cases, female 10 cases), with an average age of 49 years old.

Operation methods

Methods of lateral external ventricular drain and intraventricular perfusion of urokinase

Patients received the operation of bilateral ventricle frontal puncture and external drainage within 2h after admitted to hospital. Operation methods: The point 2.5 cm after the hairline and 2.5 cm near the medline was taken as the puncture point. The puncture direction aimed at the hypothetical line of two external ear hole, and paralleled to the sagittal plane. After the outflow of cerebrospinal fluid, the drainage tube was placed for 1.5-2.5cm. Some old hemorrhage should be removed as much as possible so as to keep the drainage unobstructed. Then the cerebrospinal fluid was released to reduce the intracranial pressure to normal range, so as to ease the patients' condition. The first cerebrospinal fluid specimen was collected. All the external ventricular drainage tube were provided by Medtronic company. After puncture, 5 ml isotonic saline solution containing 50 thousand U of urokinase was injected into the drainage tube for the first time, then the T-branch pipe was closed and external drainage bag was clamped. Then the drainage was opened after 2 h.

Postoperative treatment of external drainage

Postoperative routine treatment must be carried out to the patients such as dehydration, hemostasis, prevention of infection, protection of gastric mucosa, prevention of stress ulcer and other treatment. The highest point of the drainage tube from the puncture point must be maintained 15-20cm. CT was reexamined 24h after operation so as to clear the position of the drainage tube and the volume of residual intraventricular hemorrhage. The position of the drainage tube was adjusted timely and then urokinase was injected. Within one week after surgery, 20 thousand units urokinase were injected into the drainage tube every 12h. The drainage of the cerebrospinal fluid was opened after clamping the tube for 2h.

Continuing external drainage of cerebrospinal fluid was performed with drainage tubes about 7-10 days, and CT was reexamined to clear the remaining intraventricular blood. At the same time, the volume and appearance of cerebrospinal fluid and routine examination of cerebrospinal fluid were analyzed comprehensively. Then extubation was performed after the cerebrospinal fluid was clear. The drainage tube should be closed before the external drainage tube of lateral ventricle was pulled our. After 24 h observation, the extubation could be performed if the clinical symptoms showed no deterioration and continued to improve, with no intracranial hypertension.

Cerebrospinal fluid was collected on 8 h, 24 h, 48h, 72h and 1week respectively after onset and when the external ventricular drainage was performed within 8h after admission to the hospital, and then centrifuged for 15min in 3000 r/min to separate the cerebrospinal fluid out. For the control group, the cerebrospinal fluid was collected 1 time at lumbar puncture and preserved in -70^{o}C for testing, then determined with the ELISA method. The kit was bought from Biological Engineering Co., Ltd. The experiment was carried out strictly according to the reagent instructions, and the sample caveolin-1 value was obtained according to the standard curve drawn by the standard substance.

STATISTICAL ANALYSIS

The measurement data was represented by average ±standard deviation ($\bar{x}$±s). Difference significance test of measurement data were analyzed by one-way ANOVA method. $P<0.05$ meant that the difference was statistically significant. All the data were processed by SPSS12.0 statistical software.

RESULTS

The caveolin-1 level in cerebrospinal fluid of control group was (2.79±7.92) ng/L. Within 8 h, 24h, 48h, 72h and 1 week after onset, the caveolin-1 level in cerebrospinal fluid of severe group were all higher than that in control group ($P<0.05$). The caveolin-1 level in cerebrospinal fluid of severe group was the highest in 72 h and the lowest in 1 week after onset. Within 8 h, 24 h, 48 h, 72h and 1 week after onset, the caveolin-1 level in cerebrospinal fluid of light-medium group were all higher than that in control group, and the caveolin-1 level in cerebrospinal fluid of severe group were all higher than that in light-medium group ($P<0.05$). As shown in table 1.

DISCUSSION

The preliminary statistical results of China's brain trauma database until 2012 have shown that among the more than 13 hospitalized patients with acute traumatic brain injury in 47 hospitals in China, the mortality of patients with

Table 1: The change of caveolin-1 level in cerebrospinal fluid between the two groups (ng/L)

Groups	Within 8 h after onset	Within 24 h after onset	Within 48 h after onset	Within 72 h after onset	Within 1 week after onset
Severe group	62.53±11.80*#	67.38±19.57*#	69.52±14.72*#	70.16±10.75*#	48.11±19.67*
Light-medium group	50.28±12.99*	53.09±10.04*	55.92±10.07*	57.35±16.80*	45.82±17.58
Control group	3.795±7.928*				

Note: *compared with control group (P<0.05); #compared with light-medium group (P<0.05).

severe traumatic brain injury was more than 20%, and the rate of severe disability was more than 50% (Liu, 2011), which is similar to those reported in recent global brain trauma cases (Wang, 2014).

Caveolin-1 has a certain amount of expression in normal brain tissue. Positive cells are mainly seen in the cerebral cortex and paraventricular area with tubulous or cord-like as common form. It is an important protein to maintain the normal development and function of the brain. Research have found that the permeability of the blood brain barrier increases gradually when brain injury leads to cerebral ischemia.

The expression of caveolin in the brain mainly focus on neurons, glial cells and endothelial cells (Zhang *et al*, 2012). Caveolin-1 is related to the occurrence of blood vessels and nerve protection (Gaudreault *et al*, 2004).

Caveolin-1 has a certain relationship with inflammation-mediated tissue damage. In Caveolin-1 gene knockout mice model of acute intracerebral hemorrhage, the leukocyte infiltration is significantly reduced, the activity of matrix metalloproteinase decreased and the expression of inflammatory mediator including MIP-2 and COX-2 also reduced. In addition, Caveolin-1 deficiency can inhibit the expression of monocyte chemoattractant protein-1 (MCP-1) in endothelial cells and vascular system, and reduce the inflammatory damage by down-regulating pro-inflammatory mediators (Parat, 2009). In hemorrhagic brain tissues, the up-regulation of chemokines (such as MIP-2) can induce changes in the permeability of monocytes around the injured tissue. COX-2 can up-regulate the prostacyclin levels and promote the formation of oxygen free radicals, thus leading to brain edema and neuronal apoptosis (Chang *et al*, 2011). On the contrary, the decrease or deletion of Caveolin-1 expression can inhibit the penetration ability of local neutrophils and reduce the formation of MIP-2 and COX-2, thereby reducing the brain damage caused by hemorrhagic inflammation (Benvenisti-Zarom *et al*, 2006).

Caveolin-1 plays an important role in the phosphorylation of SRC and ERKl/2 signal transduction pathways. Its abnormal expression can destroy the membrane lipid rafts, leading to the obstruction of multiple signal transduction pathways, and ultimately promoting the nerve injury. The pathological changes of Caveolin-1 are similar to that of neurodegenerative diseases (Saleem *et al*, 2008). A variety of signal molecules are concentrated in caveolae, while caveolin-1 plays a direct negative regulation on the active state of the key molecules among them (Stary *et al*, 2012; Anderson and Jacobson, 2002).

Caveolin-1 can interact with the N-Methyl-D-Aspartate Receptor (NMDAR) to regulate NMDAR mediated neurotransmitter release and neuronal plasticity (Carver and Schnitzer, 2003).

The results of this experiment indicated that the expression of caveolin-1 was significantly increased in the cerebrospinal fluid of patients with ventric ular hemorrhage after cerebral injury within 8 h, 24 h, 48 h, 72 h and 1 week after onset. Compared with control group, the difference was significant (Gaudreault *et al*, 2005). Meanwhile, the caveolin-1 level in cerebrospinal fluid of severe group was the highest in 72h and the lowest in 1 week after onset. Within 8h, 24h, 48h and 72h after onset, the caveolin-1 level in cerebrospinal fluid of severe group were all higher than that in light-medium group (P<0.05), indicated that the expression of caveolin-1 in cerebrospinal fluid after craniocerebral injury was related to the severity of the disease.

CONCLUSION

In conclusion, the caveolin-1 level in cerebrospinal fluid after traumatic brain injury was significantly increased after intraventricular hemorrhage. It is helpful to understand the severity of the disease by detecting the index of caveolin-1 in cerebrospinal fluid. At present, caveolin-1 is mainly involved in the secondary neurotoxicity, nerve inflammation response and oxidative stress injury in hemorrhagic cerebral vascular disease. Therefore, the targeted regulation of Caveolin-1 expression and localization and interfering the formation of lacunae protein are expected to become a new approach for treatment of traumatic brain injury. And Caveolin-1 will become an important target for new drug research and development. Further study of the mechanism of Caveolin-1 in neuralprotection may lead to a new approach for the treatment of intraventricular hemorrhage due to craniocerebral injury.

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