Research Article

Chen Peng, Shibo Duan, Lou Gang*

Efficacy of Danhong injection on serum concentration of TNF-α, IL-6 and NF-κB in rats with intracerebral hemorrhage

The brain water content was significantly higher in the model group for days 3 to 9 compared to the Danhong group (P<0.05). Compared to the model group, the serum NF-κB was significantly lower in the Danhong group for the time point of day 3 and 5 (P<0.05); however, compared to the model group, the serum TNF-α and IL-6 levels in the Danhong group were significantly lower for each time point (P<0.05). Conclusion Danhong injection can reduce cerebral edema in rats with cerebral hemorrhage, and protect the brain nerve function. These effects may be related to its function of regulating serum TNF-α, NF-κB and IL-6 expression.

Keywords: Danhong injection; intracerebral hemorrhage; rats; TNF-α; interleukin 6; tumor necrosis factor alpha

1 Introduction

In recent years, with the aging of the population in China, the proportion of the elderly population has increased significantly [1]. As a result, the incidence of age related diseases including cancer, hypertension, cardiovascular and cerebrovascular diseases, have increased significantly. Hypertensive cerebral hemorrhage (HCH) is the main cause of cerebral hemorrhage. HCH accounts for 90% of the total number of patients with cerebral hemorrhage, and the incidence rate of cerebral hemorrhage is increasing year by year [2, 3]. HCH is a serious complication of hypertension. Clinical epidemiological data show that the incidence HCH is mostly concentrated in people 50 to 60 years old and the patient usually had a long history of hypertension with uncontrolled blood pressure [4, 5].

Studies have demonstrated that brain edema, which can increase intracranial pressure, is an important factor that affected the prognosis of patients with intracerebral hemorrhage [6, 7]. Inflammatory reaction is another important factor related to the prognosis of patients with
cerebral hemorrhage. Therefore, how to reduce brain edema and inflammatory reaction after intracerebral hemorrhage has become a hotspot of the present studies. Danhong injection is a compound that consists of two traditional Chinese medicines, Danshen and Honghua, which have been widely used in the treatment of stroke [8, 9]. However, its effects of neuroprotection and serum TNF-α, IL-6 and NF-κB concentration in rats with intracerebral hemorrhage had rarely been reported. In this study, we investigate the efficacy of Danhong injection on serum concentration of TNF-α, IL-6 and NF-κB in rats with intracerebral hemorrhage (ICH) and evaluate its therapeutic effects on inflammation and cerebral edema.

2 Material and methods

2.1 Animal and reagents

Seventy-five healthy SPF class Wistar male rats were purchased from Wenzhou Medical University. Rats were fed and housed at the SPF laboratory animal room. The housing condition was an ambient temperature of 20–25°C, under a 12/12 h day/night cycle, with access to food and water ad libitum. Danhong injection was purchased from Heze Buchang Pharmaceutical Co., Ltd (China); Rats serum TNF-α ELISA kits were purchased from PeproTech Co., Ltd (U.S); Rats serum IL-6 and NF-κB ELISA kits were purchased from U.S.A TSZ biological Trade Co., Ltd.

Ethical approval: The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals. All experiments were performed following Ruian People’s Hospital and the People’s Republic of China guidelines and regulations. Animal experimental procedures were approved by the Animal Ethics Committee of the Ruian People’s Hospital and performed in accordance with the guidelines for the use of experimental animals from the National Institutes of Health.

2.2 Establishment of intracerebral hemorrhage model in rats

First, rats were anesthetized with 10% chloral hydrate (35 mg/kg) through intraperitoneal injection. Then, an incision at the center of the head was made to expose the front fontanel and coronal suture. A 5 mL syringe needle was used to make a small hole at the point of 0.2 mm in front of the coronal and 3 mm next to the mid line, according to the methods described by Li et al [10]. 100 μL autologous arterial blood was injected into the brain. After blood injection, bone cement was used to seal the hole. Then, the skin of the head was sutured. Rats in the control group were given the same operation process without injection of arterial blood.

2.3 Neurological Deficit Score evaluation

The neurological deficit score (NDS) was evaluated in the three groups for each time point. 0 = no symptoms; 1 = carpal joint and elbow joint flexed, and shoulder adduction flexed; 2 = the same symptoms plus decreased muscle strength on affected side; 3 = Rat moves to one side and chases its tail; 4 = Disorder of consciousness and no spontaneous activity.

2.4 Brain water content measurement

Rats were killed using the neck breaking method on days 1, 3, 5, 7 and 9 after modeling. Perihematoma brain tissue (100 mg) was weighed and then dried by the oven at a temperature of 100 °C for 24 h, and then re-weighed. The brain tissue was dried using the same conditions. Brain water content was calculated by the formula: brain water content=(wet weight-dry weight)/wet weight×100%.

2.5 Statistical analysis

The data was expressed as mean ± standard deviation, and the difference between the three groups was tested by one-way ANOVA. LSD-t test was used for comparison between each of the two groups. The serum levels of TNF-α, IL-6 and NF-κB over different time points were compared by paired t test. P<0.05 was used to determine statistical difference. All the analyses was done using Stata 11.0 statistical software.

3 Results

3.1 Neurological Deficit Score

The neurological deficit score (NDS) of the model and Danhong groups is demonstrated in Table 1. The NDS was not statistically different for days 1, 3 and 5. However, on day 7 and 9 after modeling, the NDS in the Danhong group was significantly lower than that of the model group (P<0.05).
### Table 1. The neurological deficit scores for Model and Danhong groups

| Day | Model (n=5) | Danhong (n=5) | t   | p   |
|-----|-------------|---------------|-----|-----|
| 1   | 3.49±0.31   | 3.52±0.33     | 0.15| 0.89|
| 3   | 3.24±0.24   | 3.10±0.22     | 0.9 | 0.36|
| 5   | 3.10±0.22   | 2.77±0.26     | 2.17| 0.06|
| 7   | 2.89±0.21   | 2.41±0.21     | 3.61| 0.01|
| 9   | 2.51±0.24   | 2.12±0.19     | 2.85| 0.02|

### 3.2 Brain water content

The brain water content of the control, model and Danhong groups is demonstrated in Table 2. The brain water content in the model and Danhong groups was significantly elevated compared to the control group (P<0.05). The brain water content was significantly elevated after modeling in the model and Danhong groups for day 3 and gradually decreased over the next 6 days (Figure 1). The brain water content was significantly higher in the model group for day 3 to day 9 compared to the Danhong group (P<0.05).

### Table 2. The brain water content for control, model and Danhong groups comparison (%)

| Day | Control (n=5) | Model (n=5) | Danhong (n=5) | F   | p   |
|-----|---------------|-------------|---------------|-----|-----|
| 1   | 73.85±1.54    | 78.21±1.88  | 77.69±1.67    | 9.78| 0.003|
| 3   | 74.11±1.36    | 86.19±1.78  | 82.61±1.88*   | 66.90| <0.001|
| 5   | 73.92±1.47    | 84.20±1.65  | 76.32±1.54*   | 59.92| <0.001|
| 7   | 74.01±1.62    | 81.11±1.52  | 75.23±1.32*   | 32.38| <0.001|
| 9   | 74.32±1.42    | 77.62±1.36  | 75.34±1.47*   | 7.06 | 0.009|

*Compared to model group, P<0.05

### 3.3 Serum TNF-α, IL-6 and NF-κB concentration

The serum TNF-α, IL-6 and NF-κB concentrations were statistically different for the control, model and Danhong groups for each time point (P<0.05), Table 3. Compared to the model group, the serum NF-κb was significantly lower in the Danhong group for the time point of day 3 and 5 (P<0.05). However, compared to model group, the serum concentration of TNF-α and IL-6 in the Danhong group was significantly lower for each time point (P<0.05), Figure 2.

### 4 Discussion

Epidemiological investigation shows that the incidence of cerebral hemorrhage is the second most diagnosed cerebrovascular diseases only next to ischemic stroke [3]. The incidence rate is about 10/10 million to 15/10 million per-year. Cerebral hemorrhage has become an significant cause of death and disability. The main causes of cerebral hemorrhage are hypertension, cerebral vascular malformation, aneurysm, and arteriosclerosis. However, the exact mechanism of the disease is not fully understood. Previous publications have demonstrated...
C. Peng, et al.

Danhong injection can improve blood circulation, inhibit inflammatory reaction and promote the absorption of hematoma. The above effects of Danhong injection can improve the symptoms of neurological deficit and various other symptoms [13]. Furthermore, Danhong injection also has bidirectional function in regulation of blood circulation and hemostasis [14].

In our present study, we found that Danhong injection can reduce cerebral edema in rats with cerebral hemorrhage. Brain edema and inflammation played an important role in the development of cerebral hemorrhage and can affect the patient's prognosis [7]. Therefore, reducing brain edema and inflammation can improve the prognosis of patients with cerebral hemorrhage, theoretically.

Danhong injection, composed of danshen root (Radix Salvia Miltiorrhizae) and honghua flower (Flos Carthami Tinctorii), is widely used for cerebral hemorrhage patients clinically [11, 12]. Clinical studies have showed that Danhong injection can improve blood circulation, inhibit inflammatory reaction and promote the absorption of hematoma. The above effects of Danhong injection can improve the symptoms of neurological deficit and various other symptoms [13]. Furthermore, Danhong injection also has bidirectional function in regulation of blood circulation and hemostasis [14].

In our present study, we found that Danhong injection can reduce cerebral edema in rats with cerebral hemorrhage.

Table 3. Serum TNF-α, IL-6 and NF-κB concentration in different time points for control, model and Danhong groups.

|         | Control | Model | Danhong |
|---------|---------|-------|---------|
| NF-κB(ng/mL) |         |       |         |
| 1       | 5.2±0.18 | 16.8±2.30 | 15.6±2.70 |
| 3       | 6.2±0.19 | 25.4±4.10 | 20.2±3.10 |
| 5       | 5.9±0.16 | 22.4±3.60 | 16.2±2.50* |
| 7       | 5.4±0.17 | 18.4±3.10 | 14.1±2.10* |
| 9       | 5.1±0.14 | 15.7±2.20 | 13.0±1.90 |
| TNF-α(ng/mL) |         |       |         |
| 1       | 1.2±0.10 | 2.6±0.18 | 2.3±0.17 |
| 3       | 1.2±0.11 | 3.1±0.22 | 2.6±0.24* |
| 5       | 1.3±0.09 | 2.8±0.26 | 2.1±0.20* |
| 7       | 1.2±0.12 | 2.6±0.21 | 1.8±0.17* |
| 9       | 1.5±0.33 | 25.4±6.23 | 19.6±2.09* |
| IL-6(ng/L) |         |       |         |
| 1       | 15.5±1.89 | 29.6±3.21 | 24.4±2.69* |
| 3       | 16.9±1.45 | 33.0±6.12 | 27.1±3.41* |
| 5       | 15.6±1.32 | 30.3±3.69 | 24.1±3.04* |
| 7       | 15.2±1.47 | 28.6±3.89 | 21.1±2.47* |
| 9       | 15.4±1.33 | 25.4±6.23 | 19.6±2.09* |

*Compared to model group, P<0.05

Figure 2. Bar plot of serum TNF-α, IL-6 and NF-κB concentrations for different time points for control, model and Danhong groups.
hemorrhage, and protect the brain nerve function. This effect may be related to its function of regulating serum levels of TNF-α, NF-κB and IL-6. As previously established, TNF-α, NF-κB and IL-6 are important inflammatory mediators which are involved in multiple inflammatory reactions after intracerebral hemorrhage. TNF-α is a polypeptide cytokine with multiple biological activities, which is mainly produced by monocytes, macrophages, T lymphocytes, and microglia cells in the nervous system. It is an important mediating factor of the inflammatory response and the immune response. Cerebral ischemia and hypoxia is often found after cerebral hemorrhage, which produces a large number of antigen substances, and then stimulates monocytes, macrophages and T lymphocytes to produce a large amount of TNF-α. TNF-α then stimulates endothelial cells and macrophages to produce IL-1 and other adhesion factors, causing a cascade reaction, leading to long-lasting inflammatory responses that aggravate nerve damage. In our study, we found that rats in the Danhong group had lower concentrations of serum TNF-α, indicating that Danhong injection can reduce the expression of TNF-α in serum and protect the brain. IL-6 and NF-κB serum levels were also decreased in the Danhong group compared to the model group. These findings were in accordance with previous publications which also demonstrated that Danhong injection protected brain nerve function by regulating serum TNF-α, NF-κB and IL-6 [15, 16].

Conflict of interest: Authors state no conflict of interest.

References
[1] Liang Y., Niu X., Lu P., The aging population in China: Subjective well-being of empty nesters in rural eastern China, J Health Psychol, 2017:1359105317717599
[2] Suzuki K., Sakamoto T., Clinical epidemiology of cerebral hemorrhage, Nihon Rinsho 2006, 64 Suppl 8:315-9
[3] Pasqualin A., Epidemiology and pathophysiology of cerebral vasospasm following subarachnoid hemorrhage, J Neurosurg Sci, 1998, 42, 15-21
[4] Viale G., Aneurysmic subarachnoid hemorrhage: epidemiology and cerebral circulatory physiopathology in the acute phase, Minerva Anestesiol, 1998, 64, 115-6
[5] Franklin S.S., Hunt M.T., Vogt T., Walsh G., Paglia D.E., Hypertension and Cerebral Hemorrhage: A Malpractice Controversy: Efficacy of Drug Therapy, Epidemiology, Neurological Aspects and Pathology, Western Journal of Medicine, 1980, 133(2), 124-140
[6] Carhuapoma J.R., Hanley D.F., Banerjee M., Beauchamp N.J., Brain edema after human cerebral hemorrhage: a magnetic resonance imaging volumetric analysis, J Neurosurg Anesthesiol, 2003, 15, 230-3
[7] Lee K.R., Kawai N., Kim S., Sagher O., Hoff J.T., Mechanisms of edema formation after intracerebral hemorrhage: effects of thrombin on cerebral blood flow, blood-brain barrier permeability, and cell survival in a rat model, J Neurosurg 1997, 86, 272-8
[8] Wan J., Wan H., Yang R., Wan H., Yang J., He Y., Zhou H., Protective effect of Danhong Injection combined with Naoxintong Capsule on cerebral ischemia-reperfusion injury in rats, J Ethnopharmacol, 2018, 211, 348-357
[9] Wei J., Zhang Y., Jia Q., Liu M., Li D., Zhang Y., et al., Systematic investigation of transcription factors critical in the protection against cerebral ischemia by Danhong injection, Sci Rep, 2016, 6, 29823
[10] Li Y.B., Cui X.N., Li Y., Pan L., Wen J.Y., Effect of two Chinese medicinal compounds, blood-activating and water-draining medicine, on tumor necrosis factor α and nuclear factor κ B expressions in rats with intracerebral hemorrhage, Chin J Integr Med, 2014, 20, 857-64
[11] Li L., Quanwu G., Danhong Injection Promotion Cerebral Hemorrhage Acute Stage Haematoma Absorption Curative Effect, Journal of Practical Traditional Chinese Internal Medicine, 2009, 23, 77-78.
[12] Wenxia L., 50 Cases with Danhong Injection on Acute Hypertensive Intracerebral Hemorrhage. International Journal of Traditional Chinese Medicine, 2008, 30, 45, 77
[13] Yang M., Orgah J., Zhu J., Fan G., Han J., Wang X., et al., Danhong injection attenuates cardiac injury induced by ischemic and reperfused neuronal cells through regulating arginine vasopressin expression and secretion, Brain Res, 2016, 1642, 516-523
[14] Zhang Y.Y., Zhou H.F., Yang J.H., He Y., Chen X.Q., Nishinari K., et al., Effects of Danhong Injection and its main components on anticoagulation and fibrinolysis in cultured vein endothelial cells, Chin J Integr Med, 2016, 22, 276-83
[15] Peiyun N., Hongyong Z., Zhichao Z., Xinlin W., Fang W., Effect of Danhong injection on cerebral edema and plasma levels of MMP-9, TIMP-1 and NF-kB in rats with experimental intracerebral hemorrhage, Chinese Journal of Difficult and Complicated Cases, 2017, 16 821-824
[16] Peiyun N., Hongyong Z., Zhichao Z., Effect of Danhong Injection on serum levels of TNF-α, CRP and IL-6 in rats with experimental intracerebral hemorrhage, Chinese Journal of Clinical Research, 2017, 30, 971-973