

## ARTICLE

# Effect of Warm Acupuncture on “Dinghui Acupoint” and “Heart Acupoint” in Traditional Mongolian Medicine on Behaviors and Hypothalamic Inflammatory Cytokines in Rats with Chronic Fatigue Syndrome

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### ARTICLE INFO

#### Article history

Received: 16 January 2019

Revised: 12 February 2019

Accepted: 25 March 2019

Published Online: 1 April 2019

#### Keywords:

Warm acupuncture in Traditional  
Mongolian Medicine

Chronic fatigue syndrome (CFS)

Behaviors

Cytokines

### ABSTRACT

**Objective:** To observe the effects of warm acupuncture on “Dinghui Acupoint” and “Heart Acupoint” in Traditional Mongolian Medicine on behavior and hypothalamic inflammatory cytokines IL-1 $\beta$ , IL-6 and IFN-r in Rats with Chronic Fatigue Syndrome. **Methods:** SD rats were randomly divided into normal group, model group, warm acupuncture group and moxibustion positive control group. The latter three groups of rats were used to establish a model of rats with chronic fatigue syndrome (CFS) using a combination of physical fatigue and mental fatigue. When establishing the model of warm acupuncture group, “Dinghui Acupoint” and “Heart Acupoint” intervention was carried out; when establishing the model of moxibustion positive control group, “Zusanli Acupoint” intervention was carried out on both sides. Behavioral observations (body weight, exhaustive swimming time, tail suspension experiment, water maze) were performed before and after modeling. The hypothalamic inflammatory cytokines IL-1 $\beta$ , IL-6 and IFN-r were detected by ELISA method after warm acupuncture and moxibustion intervention. **Results:** After 21 days of modeling, the body weight of the rats in each group was significantly lower than that in the normal group, and there was a significant difference ( $P < 0.01$ ); Compared with the model group, the weight of the rats in the warm acupuncture group increased significantly, and there was a significant difference ( $P < 0.01$ ); Compared with the model group, the exhaustive swimming time of the rats in the warm acupuncture group was significantly prolonged, and there was a significant difference ( $P < 0.01$ ); Compared with the moxibustion group, the exhaustion time of the rats in the warm acupuncture group was relatively prolonged, and there was a significant difference ( $P < 0.05$ ); Compared with the normal group, the tail suspension time of the model group was significantly prolonged, and there was a significant difference ( $P < 0.05$ ); Compared with the model group, there was a significant difference in the duration of the suspension of the warm acupuncture group and the moxibustion group ( $P < 0.01$ ); Compared with the normal group, the total distance of the water maze test was shorter in the model group, and there was a significant difference ( $P < 0.01$ ); Compared with the model group, both the warm acupuncture group and the moxibustion group were prolonged, and there was a significant difference ( $P < 0.05$ ); Compared with the moxibustion group, the distance between the rats in the warm acupuncture group was relatively longer, but there was no significant difference ( $P > 0.05$ ); Compared with the normal group, IL-1 $\beta$ , IL-6 and IFN-r increased significantly in the model group and there was a significant difference ( $P < 0.05$ ); Compared with the model group, IL-1 $\beta$  and IL-6 in the warm acupuncture group was significantly decreased ( $P < 0.05$ ), and the IL-6 in the moxibustion group was significantly different ( $P < 0.05$ ); Compared with the model group, there was no significant difference between the IFN-r group and the moxibustion group ( $P > 0.05$ ); Compared with the moxibustion group, the levels of IL-1 $\beta$ , IL-6 and IFN-r were not significantly different ( $P > 0.05$ ). **Conclusion:** Warm acupuncture on “Dinghui Acupoint” and “Heart Acupoint” in Traditional Mongolian Medicine has the ability to improve the body’s defense and self-healing ability, improve chronic fatigue syndrome (CFS), and thus play a preventive role. The results of this research indicate that the warm acupuncture group and the moxibustion group have the same effect.

## 1. Introduction

Chronic fatigue syndrome (CFS) is characterized by long-term mental fatigue and physical fatigue as clinical manifestations of clinical syndrome, often accompanied by headache, sore throat, muscle and joint pain, memory loss, hypothermia, depression and other neurological symptoms, and no organic lesions. CFS is easy to occur in people between the ages of 30 and 50. The course of disease lasts for several months to several years. Although many people can continue to work, their work ability and efficiency are obviously reduced, and the symptoms of fatigue are not alleviated by rest. CFS has become a common sub-health state in modern society, and its incidence is gradually increasing. Relevant experts believe that this disease will be the “invisible killer of health” in the 21st century and is one of the main problems affecting human health. CFS is mainly caused by a variety of stressors in daily life that can trigger the body's stress response,<sup>[1]</sup> resulting in a variety of tissue and organ disorders in the body characterized by fatigue.<sup>[2]</sup> The currently accepted view is that the neuroendocrine system changes under stress, and in addition to the hypothalamic-pituitary-adrenal axis, macrophage inflammatory cytokines in the immune system are also involved in the stress response. These cytokines can affect the neuroendocrine system and work together to complete the stress response. Pall<sup>[3]</sup> and others believe that inflammatory cytokines are closely related to the pathology of patients with CFS. Western medicine has no specific drugs, mainly to relieve clinical symptoms and the treatment effect is not good. This research group has been devoted to the research of this disease from the perspective of Mongolian medicine for many years. It is concluded that Mongolian medicine warm acupuncture treatment can better improve the clinical symptoms of CFS patients.<sup>[4,5]</sup> Further research and observation based on the basic research has been carried out

to observe the mechanism of action and effect of treatment of CFS by warm acupuncture on “Dinghui Acupoint” and “Heart Acupoint”, and compare it with moxibustion in Traditional Chinese Medicine, the results are as follows.

## 2. Materials and Methods

### 2.1 Animals and Grouping

SD male rats, clean grade, weighing 160-180g, 48 rats, provided by Beijing Weitong Lihua Animal Experiment Co., Ltd. (license number: SCXK (Beijing) 2016-0011). Forty-eight rats were weighed and weighed from small to large. They were divided into four groups, 12 rats in each group, and then 48 pairs of random numbers were used to match four groups. According to the pairing numbers, the group of 3 groups in each group from small to large was divided into four groups, namely normal group, model group, warm acupuncture group and moxibustion group, 12 in each group. The indoor temperature is maintained at 20-26 ° C, the relative humidity is 40%-70%, and the feeding is adapted for 3 days before the experiment, free diet and feeding.

### 2.2 Main Equipment

MYL-I type temperature needle instrument: Inner Mongolia Yuanyang Zhongmeng Medical Technology Development Co., Ltd. Special silver needle: 0.5\*3cm, Ai grain: 7mm\*8mm is provided by Beijing Zhongtitai Medical Co., Ltd. Suspension box: height 60cm width 40cm depth 50cm, according to the relevant literature. Water Maze Instrument: WMT-100, provided by Chengdu Taimeng Technology Co., Ltd. Bucket: 100cm deep and 50cm in diameter, stopwatch, electronic scale, thermometer, etc.

### 2.3 Model Preparation

After adapting to the environment for 3 days, the model

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Fund Project:

Funded by National Natural Science Foundation of China (Project No.: 81560802).

group, warm acupuncture group and moxibustion group rats were placed in a bucket for 3 days of swimming, once a day, once for 20 minutes, and two rats were bathed once a bucket, the water depth was 80 cm. The water temperature is  $30\pm 2^{\circ}\text{C}$ . Then on the fourth day, the official weight-bearing swimming (weight is 5% body weight, body weight is weighed every 3 days) for 21 days. When the rat does not move on the surface of the water, it is driven by a wooden stick to maintain its exercise state. Exhaustive standard: the swimming movement is obviously out of tune, and can no longer be adhered to. When the nose sinks into the water for 5 seconds, it cannot be returned to the surface of the water as exhaustion. When the animals appear exhausted, they should be picked up in time, dried with a cloth, and rested in a cage.

## 2.4 Methods

Regular feeding in the normal group; the model group was modeled according to the preparation conditions. The warm acupuncture group “Dinghui Acupoint” and “Heart Acupoint” were treated alternately with warm acupuncture every other day. The two points are obliquely inserted into the needle, and the special acupuncture needle of Mongolian medicine is used, the depth is 0.5-1.0cm, the temperature is 100mA, and the temperature is  $40^{\circ}\text{C}$ . Each time 20min, the course of treatment is 21 days, Dinghui Acupoint 6 times, Heart Acupoint 5 times, a total of 11 times. The moxibustion group was treated with moxibustion on both sides of the Zusanli, 20 minutes each time, 3 times on one side, 6 times in total, the course of treatment was 21 days, and the treatment was performed every other day for 11 times.

## 2.5 Behavioral Detection Method

During the modeling period, the rats were generally weighed on the 21st day, the swimming exhaustion time was recorded, and the tail suspension experiment and the water maze test were performed. The hanging tail experiment method was to stick with a medical tape 1 cm away from the tail end of the rat, and hung in the center of the tail box to observe the immobility time within 5 minutes. The water maze experiment recorded the distance traveled in the sink in 3 minutes.

## 2.6 Material Drawing and Testing

On the 22nd day after the behavioral test of each group of rats, the rats were fasted for 24 hours, the hypothalamus was taken off the head, and the tissue was placed in a cryogenic vial for testing. The levels of IL-1 $\beta$ , IL-6 and IFN- $\gamma$  in the hypothalamus were detected by ELISA and

operated according to the instructions of the ELISA kit.

## 2.7 Statistical Methods

SPSS22.0 statistical software was used for data processing. The data values of measurement data were expressed by  $\bar{X}\pm S$ . One-way analysis of variance was used among multiple groups. The homodyne LDS and the variance of Dunnett T3 were compared with  $P < 0.05$ .

## 3. Results

### 3.1 General Situation

There were no significant differences in feed intake, water intake, body weight, and fur gloss of the four groups before the experiment. After the experiment, the normal group showed normal behavior and the body weight increased significantly compared with that before the experiment. The rats in the model group showed typical “fatigue” characteristics before the experiment: the mental state was not good, the drinking water was small, the stool was not formed, the fur fell off, the gloss is obviously reduced, the activity is reduced, and the blinking is lazy, lethargy, irritability, unresponsiveness, and significant weight loss. The amount of food and water in the warm acupuncture group and the moxibustion group were generally lower than those in the normal group and increased compared with the model group. In the middle of the two groups, the skin gloss was poor and the stool was normal.

### 3.2 Changes in Behavior of Rats in Each Group

#### 3.2.1 Comparison of Body Weight before and after the Experiment in Each Group of Rats (see Table 1)

**Table 1.** Comparison of Body Weight before and after the Experiment in Each Group of Rats (Units: g,  $\bar{X}\pm S$ )

Group	N	1 <sup>st</sup> Day	21 <sup>st</sup> Day
Normal Group	10	209.36 $\pm$ 8.53	323.60 $\pm$ 11.57
Model Group	10	212.91 $\pm$ 8.10	304.70 $\pm$ 6.52**
Warm Acupuncture Group	10	211.00 $\pm$ 8.18	328.10 $\pm$ 9.48##
Moxibustion Group	10	208.58 $\pm$ 8.28	317.40 $\pm$ 23.04
F		0.93	5.18

**Notes:** \*\* $P < 0.01$  compared with the normal group, ## compared with the model group,  $P < 0.01$ .

It can be seen from Table 1 that there was no significant difference in body weight between the groups before the model establishment ( $P > 0.05$ ). Compared with the normal group, the weight of the model group was significantly lower than that of the normal group ( $P < 0.01$ ). Compared with the model group, the weight of the warm acupuncture

ture group increased significantly, and there was a significant difference ( $P < 0.01$ ); Compared with the moxibustion group, the weight of the warm acupuncture group was relatively increased, but there was no significant difference ( $P > 0.05$ ).

### 3.2.2 Comparison of Exhaustion Time before and after the Experiment in Each Group of Rats (see Table 2)

**Table 2.** Comparison of Exhaustion Time before and after the Experiment in Each Group of Rats (Units: s,  $\bar{X} \pm S$ )

Group	N	1 <sup>st</sup> Day	21 <sup>st</sup> Day
Model Group	10	858.58±165.24	201.00±87.88
Warm Acupuncture Group	10	884.83±499.23	639.90±378.07** <sup>△</sup>
Moxibustion Group	10	857.83±318.01	348.40±188.90#
F		0.02	8.03

**Notes:** \*\* Compared with the model group,  $P < 0.01$ , # compared with the warm acupuncture group,  $P < 0.05$ , <sup>△</sup> compared with the moxibustion group,  $P < 0.05$ .

Since the normal group did not perform adaptive swimming, the swimming data was not exhausted. It can be seen from Table 2 that there was no significant difference in exhaustive time between the groups before the model establishment ( $P > 0.05$ ). Compared with the model group, the swimming time of the warm acupuncture group was significantly prolonged, and there was significant difference ( $P < 0.01$ ). Compared with the moxibustion group, the swimming time of the warm acupuncture group was relatively extended. And there was a significant difference ( $P < 0.05$ ).

### 3.2.3 Comparison of the Tail Suspension Time before and after the Experiment in Each Group of Rats (see Table 3)

**Table 3.** Comparison of the Tail Suspension Time before and after the Experiment in Each Group of Rats (Units: s,  $\bar{X} \pm S$ )

Group	N	1 <sup>st</sup> Day	21 <sup>st</sup> Day
Normal Group	10	50.33±34.39	81.20±20.24
Model Group	10	49.50±22.06	116.40±63.36*
Warm Acupuncture Group	10	47.17±22.04	62.20±16.38##
Moxibustion Group	10	48.50±18.82	51.60±35.04##
F		0.39	5.48

**Notes:** \* $P < 0.05$  compared with the normal group, ## compared with the model group,  $P < 0.01$ .

It can be seen from Table 3 that there was no significant difference in the time of suspension of the rats in each group before modeling ( $P > 0.05$ ). Compared with the normal group on the 21st day of modeling, the immobil-

ity time of the model group was significantly prolonged, and there was significant difference ( $P < 0.05$ ). Compared with the model group, the warm acupuncture group and the moxibustion group had no significant movement time. Shortened, there was a significant difference ( $P < 0.01$ ).

### 3.2.4 Comparison of the Total Distance of the Water Maze before and after the Experiment in Each Group of Rats (see Table 4)

**Table 4.** Comparison of the Total Distance of the Water Maze before and after the Experiment in Each Group of Rats (Units: cm,  $\bar{X} \pm S$ )

Group	N	1st Day	21st Day
Normal Group	10	2960.76±801.66	2277.70±233.02
Model Group	10	2905.62±517.25	1597.57±244.84**
Warm Acupuncture Group	10	3129.17±501.47	2154.02±468.01#
Moxibustion Group	10	3040.21±866.73	2003.53±251.19#
F		0.24	8.83

**Notes:** \*\* $P < 0.01$  compared with the normal group, # $P < 0.05$  compared with the model group.

It can be seen from Table 4 that there is no significant difference in the total distance of water maze between the groups before the modeling ( $P > 0.05$ ). Compared with the normal group on the 21st day of modeling, the total distance of the rats in the model group was significantly reduced ( $P < 0.01$ ). Compared with the model group, the total distance of the warm acupuncture group and the moxibustion group increased. There was a significant difference ( $P < 0.05$ ).

### 3.2.5 Comparison of Inflammatory Cytokines in Hypothalamic Tissue of Rats in Each Group (see Table 5)

**Table 5.** Comparison of Inflammatory Cytokines in Hypothalamic Tissue of Rats in Each Group (Units: pg/ml,  $\bar{X} \pm S$ )

Group	N	IL-1 $\beta$	IL-6	IFN- $\gamma$
Normal Group	10	30.66±17.71	113.36±57.47	45.67±18.09
Model Group	10	58.48±21.02*	159.70±44.17*	72.17±16.22*
Warm Acupuncture Group	10	33.29±7.53#	112.98±30.58#	56.02±28.33
Moxibustion Group	10	36.85±13.19	113.96±34.56#	54.92±22.01
F		6.54	2.90	2.32

**Notes:** \* $P < 0.05$  compared with the normal group, # $P < 0.05$  compared with the model group.

It can be seen from Table 5 that the levels of IL-1 $\beta$ , IL-6 and IFN- $\gamma$  in the hypothalamus of the model group were significantly increased compared with the normal group on the 21st day of modeling, and there was a sig-

nificant difference ( $P < 0.05$ ). Compared with the model group, the levels of IL-1 $\beta$  and IL-6 in the warm acupuncture group were significantly lower, there was a significant difference ( $P < 0.05$ ), and there was no significant difference in IFN- $\gamma$  content ( $P > 0.05$ ). Significant difference ( $P < 0.05$ ); there was no significant difference in the warm acupuncture group compared with the moxibustion group.

#### 4. Discussion

In the modern efficient and fast-paced lifestyle and high-intensity work competition environment, not eating or drinking too much on time, preferring cold, spicy things, or emotional disorders, overwork, lack of exercise, and long-term physical and mental activity Such causes cause a decrease in the body's basal metabolic rate and chronic inflammation. In recent years, it has been pointed out that patients with CFS have abnormal immune function, and cytokines are closely related to the appearance of CFS symptoms. It is believed that chronic immune activation can lead to increased release of cytokines, and highly expressed pro-inflammatory cytokines disrupt neurotransmitter function, resulting in CFS fatigue, muscle pain, sore throat, swollen lymph nodes and other symptoms appear.<sup>[6]</sup> IL-1 $\beta$  and IL-6 are among the most important cytokines and play an important role in the bidirectional regulation of neuro-endocrine-immune system stress response. IL-1 $\beta$  regulates adrenocorticotrophic hormone in the hypothalamus in a stress response, and can also directly act on the pituitary to promote the secretion of adrenocorticotrophic hormone. Conversely, glucocorticoids can also reverse the secretion of IL-1 $\beta$ .<sup>[7]</sup> The research found that normal rats do not contain IL-1 $\beta$  in the blood or the content is very low, and elevated IL-1 $\beta$  indicates tissue damage or infection in the body.<sup>[8]</sup> IL-6 is considered to be a fatigue-inducing cytokine. The pro-inflammatory factors IL-1 $\beta$ , IL-6 and IFN- $\gamma$  are involved in the formation of chronic inflammation and can trigger fatigue, depression and flu-like symptoms in patients with similar CFS. IL-6 is mainly involved in adaptive immune regulation and is affected to some extent by IL. The effect of -1 $\beta$  secretion, while elevated IL-6 levels were observed in most chronic inflammation, showing that IL-6 response may be more pronounced in chronic inflammation caused by chronic stress.<sup>[9,10,11]</sup> IFN- $\gamma$  is a multi-functional cytokine produced by activated T lymphocytes, which can enhance the cytotoxic activity of natural killer cells (NK), activate phagocytosis of macrophages, and enhance the body's non-specific immune system. Functional disease resistance, which in turn promotes B cell differentiation, produces antibodies, ultimately kills microorganisms, and plays an important role in antiviral and immune function.

IL-1 $\beta$ , IL-6 and IFN- $\gamma$  act as pro-inflammatory cytokines not only involved in the regulation of body fever, feeding, sleep, arousal, etc., but also induce mental and neurological symptoms such as apathy, fatigue, and delusions.

Traditional Mongolian Medicine believes that the occurrence and development of diseases are always closely related to psychological factors, social factors, environmental factors and physical factors. Mongolian Medicine categorizes chronic fatigue syndrome in the category of Badakan and Hershey diseases, and believes that Badakan in the "Three Roots" (Heyi, Sheila, Badakan) is in a state of peace with Hershey, Heyi and Badagan are dysfunctional, causing a series of syndromes such as persistent or recurrent fatigue.<sup>[12]</sup> Therefore, in clinical practice, the disease is often treated by inhibiting the increase of "Hervey" and performing the function of Badakan. Traditional Mongolian Medicine warm acupuncture therapy has the effects of improving blood circulation, reducing inflammation and relieving pain, relieving cold and dispelling cold, regulating Badagan, Heri dysfunction, and improving the body's disease resistance. Modern medical experiments have shown that the in vitro needle temperature measured by heating the silver needle tail is about 100°C, the needle body temperature in the skin is 55°C, and the tip temperature is 39-41°C.<sup>[13]</sup> This kind of thermal energy and acupuncture stimulation is an effective means to stimulate the body to produce benign stress. It can activate the endogenous protection mechanism of the body, adjust the body, reduce or resist the damage of subsequent diseases, and achieve the protection of the body. Based on the theory of Mongolian medicine and the concept of syndrome differentiation caused by three (Hervey, Sheila, Badakan) disorders, this experiment puts forward the intervention of CFS Mongolian doctor warm acupuncture to explore the possible mechanism and effect of Mongolian doctor warm acupuncture on CFS rats.

#### 5. Conclusion

The results of this research indicate that in this experiment, the exhaustive swimming time of the model group was significantly shortened and the weight was reduced on the 21st day after exhaustive (weight-bearing) swimming modeling. The total distance of the water maze test was significantly shortened, and the suspension time was significantly prolonged. (Compared with the normal group, the model group had significant differences ( $P < 0.05$ ,  $P < 0.01$ ), and it was this change that corroborated the success of the model. The warm acupuncture group and the moxibustion group were exhausted. The swimming time, the water labyrinth experiment, the suspension time and the model group were significantly different, and there

was statistical significance ( $P < 0.05$ ), indicating that the warm acupuncture treatment group and the moxibustion treatment group all reached a certain degree of fatigue relief. To reduce the damage caused by fatigue to the immune system. The results of this experiment also indicated that the levels of IL-1 $\beta$ , IL-6 and IFN- $\gamma$  in the hypothalamus of the model group were significantly increased ( $P < 0.05$ ,  $P < 0.01$ ); compared with the model group, the levels of IL-1 $\beta$  and IL-6 in the warm acupuncture group and the moxibustion group were significantly lower ( $P < 0.05$ ), and there was no significant difference in IFN- $\gamma$  content ( $P > 0.05$ ); there was no significant difference in the warm acupuncture group compared with the moxibustion group, which indicates that warm acupuncture treatment as an effective means of benign stress, inhibit the release of cytokines, improve the disease resistance and self-healing ability of rats, reduce or resist the damage of rats after fatigue, and thus play a preventive and therapeutic role. This may be one of the mechanisms by which the Mongolian doctor warm acupuncture therapy intervenes in CFS. The results of this research also indicate that the effect of warm acupuncture therapy is not better than that of moxibustion; further research is needed.

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