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Effect of the Haoqingdingdan decoction on damp–heat syndrome in rats with influenza viral pneumonia

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ABSTRACT

Objective: To investigate the effect of Chinese medicine prescription–Haoqingdingdan decoction on damp–heat syndrome in rats with influenza viral pneumonia and its influence on the immune function. **Methods:** A total of 48 Wistar rats were randomly divided into the normal control group, the damp–heat syndrome model group, the Haoqingdingdan decoction group (high, medium and low dose group) and the ribavirin group. The body temperature and weight of rats in each group were recorded after modeling. After treatment for 6 d, the concentration of T lymphocyte subgroup (CD3⁺CD4⁺, CD3⁺CD8⁺) was determined by flow cytometry. The OD value of IFN– γ /IL–4 was detected by double–antibody sandwich ELISA method, and its concentration was acquired through conversion. **Results:** After modeling, the temperature and weight of rats in each modeling group showed the increasing trend ($P < 0.01$). From the second day of treatment, there was significant difference in the body mass between groups, and the rat weight of the control group was higher than in the modeling group ($P < 0.05$ or 0.01). With the advances of treatment, only the temperature in the medium and high dose Haoqingdingdan decoction groups declined significantly ($P < 0.05$). After treatment, the CD4⁺/CD8⁺ ratio of the damp–heat syndrome model group decreased more significantly compared with the control group. Elevated CD3⁺CD8⁺ percentages and declined CD4⁺/CD8⁺ ratios can be observed in the low dose group and ribavirin group ($P < 0.05$). Moreover, the CD3⁺CD4⁺ percentage of ribavirin group was lower than in the control group ($P < 0.05$). After treatment, the IFN– γ and IFN– γ /IL–4 levels in the peripheral blood of rats in the damp–heat syndrome group were obviously higher than in the control group ($P < 0.05$). **Conclusions:** Compared with ribavirin, the high dose Haoqingdingdan decoction can improve the ratio of T lymphocyte subgroup and Th1/Th2 cell balance more effectively.

1. Introduction

Sourced from “Renewed Popular Treatise on Febrile Diseases” Haoqingdingdan decoction is a famous

prescription founded by Gen–Chu Yu, a famous doctor in Qing dynasty[1]. This prescription was designed originally for Shaoyang damp–heat syndrome and proved to have good efficacy in clinical practice[2]. The damp–heat syndrome is an acute exogenous febrile disease caused by pathogenic dampness. The causes of damp–heat syndrome are multifactorial, including not only environmental factors, but also autoimmune function and pathogenic microorganism[3]. Viral infection is one of the common causes of damp–heat syndrome. It was found that people tend to have viral damp–heat syndrome in hot and humid environment, and

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influenza virus infection is most common^[4]. Due to the limitation of Western medicine and the hysteresis of vaccine development, the treatment of this disease is not satisfactory. However, traditional Chinese medicine shows its unique advantages in the treatment of this disease. A study found that Haoqingdan decoction can decrease the mRNA level of NF- κ B and the percentage of Th1/Th2 through decreasing the expression of TLR2, decrease lung index and improve pathological change^[5]. On the basis of related studies, we illustrated the advantages of Haoqingdan decoction in treating this disease and its role in regulating immunity through the comparison between the high, medium and low dose Haoqingdan decoction groups and the ribavirin group, which would provide basis for differential traditional Chinese medicine treatment of upper respiratory viral infection^[6].

2. Materials and methods

2.1. Experimental materials

Experimental animals and strains: 60 Wistar rats in SPF grade with the weight of 180–200 g; the influenza virus A1 (H1N1) FM1 strains were provided by the National Institute for the Control of Pharmaceutical and Biological Products). Medicine: Haoqingdan decoction. Composition: 10 g *Artemisia annua* L, 15 g baical skullcap root, 6 g tangerine peel, 10 g rhizoma *Pinelliae praeparatum*, 10 g *Fructus aurantii*, 10 g *Bambusae caulis im taeniam*, 30 g talcum, 15 g *Folium isatidis* and 6 g *Glycyrrhiza uralensis*. The decoction method referred to the Great Dictionary of TCM formulas. The medicine was concentrated to 1 g/mL and saved in the refrigerator at 4 °C. Positive control medicine: ribavirin purchased from the Department of Pharmacy of Zhengzhou Central Hospital.

2.2. Modeling and grouping

According to the method reported in the study^[7], 48 rats were randomly divided into 6 groups with 8 rats in each group, namely, the normal control group, the damp–heat syndrome model group, the high, medium and low dose Haoqingdan decoction groups (the high, medium and low dose groups for short) and the ribavirin group. The modeling started after 1d adaptive feeding. The modeling groups were raised by high–fat diet every day, while the normal group was given normal diet. On the tenth day of modeling, the rats in the modeling groups were infected by the H1N1 virus through nasal drip. 24 h after viral infection, the rats in modeling groups were put into the artificial temperature chamber at (32±0.5) °C, 55%–60% air humidity for 3 h. These rats were exposed in this environment for 3h every other day with a total heat exposure for three times. The normal control

group and modeling group were raised separately. The body weight and temperature of rats were measured at 9 am every day after modeling.

On the 17th day after modeling, intragastric administration once a day was performed for each treatment group (high, medium and low dose groups and ribavirin group). The dosage of Haoqingdan decoction was calculated according to the body surface area of human and rat. The doses of high, medium and low groups were 36.92 mg/kg/d, 18.46 mg/kg/d and 9.23 mg/kg/d, respectively. The ribavirin dose was 70 mg/kg/d. The normal control group and damp–heat syndrome model group were intragastrically administrated by normal saline.

2.3. Detection of T cell subgroup and cytokine level of rats in each group

After medication for 6 d, eyeballs were extracted for blood, and food and water were prohibited 4 h before the blood extraction. A total of 0.5 mL blood was added into the anticoagulant tube and fully mixed. Detection of T lymphocyte subgroup was finished in the same day. The serum was separated after blood standing and then was used for cytokine detection.

Detection of T lymphocyte subgroup: 0.5 mL heart blood was taken from the rats. The number and percentage of each T lymphocyte subgroup was measured by flow cytometry using three kinds of antibodies labeled by different fluorescent dyes. The operative procedures were strictly in accordance with the instruction.

Detection of the cytokine level (IFN- γ /IL-4): about 1mL supernatant was taken from rats. Double–antibody sandwich ELISA method was used to detect the concentration of cytokine. The curve was drawn using the concentration of the standard preparation as the X-axis and OD value as the Y-axis. The concentration of the specimen can be found in the curve by its OD value. IL-4/IFN- γ = concentration in the standard curve \times dilution multiple.

2.4. Statistics analysis

ANOVA and *t* test were used for experimental data. Multi-group comparisons used One–Way ANOVA. Results were considered to be statistically significant when $P < 0.05$. SPSS 16.0 software was used for statistical analysis.

3. Results

3.1. Temperature and weight changes of rats in each group after modeling

From the first day to the 14th day after modeling, the weight of rats in each experimental group and control group

increased significantly ($P<0.01$), but the rat weight difference among groups was not significant at the same time ($P>0.05$). Changes of body temperature: From the first day to the 14th day after modeling, the temperature change of the control group was not significant ($P>0.05$), but the rat temperature of the modeling groups (the damp-heat syndrome model group, the Haoqingdan decoction groups and the ribavirin group) showed the trend of increasing ($P<0.01$), and the temperature comparison at the same time showed that the temperature of

rats in the modeling groups was significantly higher than in the control group on the 14th day of modeling ($P<0.05$, Table 1, 2).

3.2. Temperature and weight changes of rats in each group after treatment

The 17th day after modeling was regarded as the first day of treatment. The intragastric administration was given

Table 1

Body weight changes of rats in each group after modeling (mean±SD, g).

Group	1 d	7 d	10 d	14 d	F	P
Control	192.4±9.1	248.8±7.6	260.6±6.5	279.8±8.5	177.3	0.00
Damp-heat syndrome model	197.5±9.0	252.7±10.5	267.3±7.8	270.7±6.5	126.2	0.00
High dose group	191.4±11.3	247.8±6.7	264.2±5.8	268.1±7.8	108.5	0.00
Medium dose group	196.7±8.6	254.1±10.0	270.6±8.3	272.3±7.1	115.4	0.00
Low dose group	187.8±8.2	246.4±8.2	263.1±9.0	266.4±8.3	120.9	0.00
Ribavirin	190.6±7.4	250.3±7.9	268.9±8.2	271.6±9.7	132.7	0.00
F	0.7	0.2	1.1	1.2		
P	0.60	0.91	0.40	0.24		

Table 2

Temperature changes of rats in each group after modeling (mean±SD, °C).

Group	1 d	7 d	10 d	14 d	F	P
Control	37.7±0.2	37.6±0.3	37.8±0.2	37.8±0.3	0.6	0.62
Damp-heat syndrome model	37.8±0.2	37.9±0.3	38.3±0.4	38.9±0.5	5.9	0.02
High dose group	37.8±0.3	38.0±0.3	38.3±0.5	39.0±0.5	4.8	0.03
Medium dose group	37.7±0.3	37.9±0.4	38.2±0.4	39.0±0.4	7.0	0.01
Low dose group	37.9±0.4	38.1±0.3	38.5±0.3	39.2±0.5	7.1	0.01
Ribavirin	37.8±0.4	38.0±0.3	38.4±0.4	39.1±0.4	7.0	0.01
F	0.6	1.5	1.6	6.0		
P	0.62	0.29	0.27	0.02		

Table 3

Weight changes of rats in each group after treatment (mean±SD, g).

Group	1 d	2 d	3 d	4 d	5 d	6 d	F	P
Control	287.6±7.3	293.8±9.0	299.4±8.1	302.5±7.8	304.4±6.8	307.8±9.2	3.5	0.04
Damp-heat syndrome model	271.1±8.5	270.0±8.3	269.0±8.8	267.5±9.0	267.9±10.2	268.1±9.7	0.1	0.98
High dose group	268.0±7.8	269.5±7.9	270.8±8.3	271.9±7.3	272.8±8.4	274±6.8	0.2	0.94
Medium dose group	271.6±8.1	272.1±8.2	272.9±7.5	273.3±8.3	273.6±8.4	274.2±8.7	0.3	0.91
Low dose group	267.5±8.5	267.8±8.0	270.2±8.9	270.8±8.8	271.5±9.0	272.0±8.9	0.2	0.97
Ribavirin	271.9±6.9	272.0±7.2	272.2±7.3	272.3±6.8	272.5±7.1	272.9±7.4	0.2	0.97
F	2.5	4.1	5.9	7.5	7.9	8.7		
P	0.09	0.02	<0.01	<0.01	<0.01	<0.01		

Table 4

Temperature changes of rats in each group after treatment (mean±SD, °C).

Group	1 d	2 d	3 d	4 d	5 d	6 d	F	P
Control	37.7±0.3	37.6±0.5	37.8±0.5	37.9±0.6	37.8±0.4	37.8±0.5	0.2	0.98
Damp-heat syndrome model	38.8±0.4	38.9±0.4	39.0±0.6	39.2±0.5	38.9±0.5	38.8±0.6	0.4	0.80
High dose group	39.0±0.3	38.8±0.5	38.6±0.4	38.3±0.6	38.1±0.5	37.8±0.4	3.7	0.03
Medium dose group	39.1±0.5	38.9±0.4	38.8±0.6	38.7±0.4	38.5±0.6	38.1±0.5	3.5	0.04
Low dose group	39.2±0.3	39.1±0.5	38.9±0.4	38.9±0.5	38.7±0.6	38.5±0.5	1.2	0.38
Ribavirin	38.9±0.4	38.9±0.5	38.8±0.5	38.7±0.3	38.7±0.3	38.6±0.4	0.5	0.76
F	7.3	3.8	2.7	3.2	2.2	3.2		
P	<0.01	0.03	0.08	0.04	0.12	0.04		

to the Haoqingdan decoction group and the ribavirin group, while the normal control group and the damp–heat syndrome model group were intragastrically administrated by normal saline. The results indicated that the weight of rats in each modeling group slowly increased, but not obviously. The group comparison indicated that from the second day of treatment, the weight difference among each group was significant, and the body weight of rats in the control group was obviously higher than in the modeling groups ($P < 0.05$ or 0.01). With the advances of treatment, obvious temperature decrease can only be observed in the medium and high dose Haoqingdan decoction groups ($P < 0.05$, Table 3, 4).

3.3. Comparison of T lymphocyte subgroup and IFN- γ /IL-4 level

After treatment for 6 days, the CD3⁺CD4⁺ percentage and CD4⁺/CD8⁺ ratio in the damp–heat syndrome model obviously decreased compared with the normal control group, but the percentage of CD3⁺CD8⁺ increased ($P < 0.05$). Increased CD3⁺CD8⁺ percentage and decreased CD4⁺/CD8⁺ ratio can be observed in the low dose group and ribavirin group ($P < 0.05$). Moreover, the CD3⁺CD4⁺ percentage of the ribavirin group was lower than in the control group ($P < 0.05$, Table 5). The IFN- γ and IFN- γ /IL-4 levels in the peripheral blood of rats in the damp–heat syndrome model was obviously higher than in the control group ($P < 0.05$, Table 6).

Table 5

Comparison of T lymphocyte subgroup in each group (mean \pm SD).

Group	CD3 ⁺ CD4 ⁺ (%)	CD3 ⁺ CD8 ⁺ (%)	CD4 ⁺ /CD8 ⁺
Control	75.0 \pm 1.7	24.1 \pm 1.3	3.1 \pm 0.4
Damp–heat syndrome model	62.5 \pm 4.1 [△]	28.2 \pm 2.3 [*]	2.2 \pm 0.2 [△]
High dose group	74.5 \pm 2.6	24.5 \pm 2.1	3.0 \pm 0.3
Medium dose group	73.9 \pm 2.5	24.9 \pm 1.9	2.9 \pm 0.3
Low dose group	73.0 \pm 3.1	25.4 \pm 3.7 [*]	2.8 \pm 0.4 [*]
Ribavirin	72.8 \pm 4.3 [*]	25.6 \pm 3.0 [*]	2.8 \pm 0.2 [*]

[△]: Compared with the control group, $P < 0.01$; ^{*}: Compared with the control group, $P < 0.05$.

Table 6

Comparison of Th1/Th2 level in each group (mean \pm SD).

Group	IFN- γ (ng/L)	IL-4 (ng/L)	IFN- γ /IL-4
Control	7 454.3 \pm 328.9	3 695.8 \pm 328.5	2.0
Damp–heat syndrome model	7 996.3 \pm 156.4 [*]	3 506.2 \pm 187.9	2.3 [*]
High dose group	7 505.8 \pm 286.7	3 490.8 \pm 217.3	2.1
Medium dose group	7 497.4 \pm 202.8	3 517.9 \pm 231.7	2.1
Low dose group	7 488.5 \pm 227.0	3 548.6 \pm 184.6	2.1
Ribavirin	7 483.6 \pm 226.5	3 601.4 \pm 198.2	2.0

^{*}: Compared with the control group, $P < 0.05$.

4. Discussion

In modern medicine, respirovirus is a kind of virus

invading into the human body through respiratory tract and causing respiratory tract and/or systemic infection. In most circumstances, this kind of virus would infect host cell and induce immune response after entering human body through respiratory tract[8]. Due to the high mutation rate, this kind of virus (such as influenza virus, parainfluenza virus, coronavirus and some enteric viruses) often brings great difficulty to clinical diagnosis and treatment in clinical practice[9,10]. The treatment of this kind of virus is mainly symptomatic treatment in modern medicine. Despite of the occurrence of many broad–spectrum antivirus agents and vaccine, this kind of infection can hardly be radically cured for the high variability and drug resistance of virus[11–14]. In the treatment of these diseases, traditional Chinese medicine focuses on the relationship between virus, organism and medicine rather than the virus itself.

Respiratory viral infection and its pathogenesis are closely related to the immune state of organism. This kind of infection belongs to the damp–heat febrile disease, because its clinical manifestation and occurrence season and region are very similar to the defensive qi & body surface and qi phase syndrome of febrile disease[15]. Having the function of Qingdan dampness, and regulating stomach and dissipating phlegm, Haoqingdan decoction is a representative prescription for clearing heat and eliminating dampness. Febrile disease theory holds that clearing heat and eliminating dampness method has the function of guiding qi downward, activating spleen, regulating stomach, promoting diuresis, clearing heat and dissipating dampness, which is suitable for damp–heat febrile disease[16]. The experimental results of our study indicated that Haoqingdan decoction can reverse the decline tendency of CD4⁺/CD8⁺ ratio caused by the synthetic action of damp–heat environment and viral infection, making the CD4⁺/CD8⁺ ratio close to the normal control group. In the comparison of Th1/Th2 cytokine (IFN- γ /IL-4) level, after treated by Haoqingdan decoction for 6 days, the IFN- γ and IFN- γ /IL-4 levels in the peripheral blood of rats in the damp–heat syndrome model group was obviously higher than in the control group, while the IFN- γ /IL-4 levels of the other modeling groups were close to the control group. These results indicated that Haoqingdan decoction can strengthen the effect of rat helper T cell, regulate the balance of T lymphocyte subsets and the balance of Th1/Th2 and strengthen the anti–virus immune function of organism[7], and the efficacy of high dose drugs was lower than the low dose. The possible mechanism of the antiviral immune injury of Haoqingdan decoction is up–regulating cellular immune function, re–balancing the CD4⁺/CD8⁺ and Th1/Th2 and lightening the stress state[17].

In the improvement of rat temperature, obvious decline

of temperature can only be observed in the medium and high dose Haoqingqingdan decoction groups ($P < 0.05$), and the effect of decreasing rat temperature was not obvious in low dose Haoqingqingdan decoction group and the ribavirin group ($P > 0.05$), which indicated that the therapeutic effect of Haoqingqingdan decoction on fever caused by influenza virus was notable^[18–20]. In the aspect of weight, although the immune state and temperature of rats in each treatment group were improved, the body weight did not significantly increase after treatment ($P > 0.05$). This indicated that damp-heat syndrome had great impact on the dietary intake and metabolism of rats, and this impact can hardly recovered in a short time. It was also found that in regulation of the balance of T cell subset ($CD4^+/CD8^+$), the therapeutic effect of ribavirin was poorer than the medium and high dose Haoqingqingdan decoction, but was close to the low dose Haoqingqingdan decoction, which indicated that ribavirin has a certain effect on the treatment of damp-heat syndrome caused by influenza virus, but is not superior to Haoqingqingdan decoction.

Conflict of interest statement

We declare that we have no conflict of interest.

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