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Analysis of the Clinical Effect of Bad Cold Resistance Mixture on Exogenous High Fever Animal Model

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Key words: exogenous high fever, bad cold resistance mixture, animal model, febrile medium

Abstract: Objective: Analyzing the function of bad cold resistance mixture on exogenous high fever of young mice febrile medium and discussing the characteristics and security of bad cold resistance mixture abatement of fever to provide clinical basis for comprehensive exploitation and utilization of bad cold resistance mixture capsule. Methods: Through the lipopolysaccharide (LPS) intraperitoneal injection to build exogenous high fever animal model and measure the temperature changes in different time points of animal model, besides, measure the interleukin 1α（IL-1α）, total white blood cell number in peripheral serum to learn the antifebrile effect of bad cold resistance mixture. Result: For bad cold resistance mixture of 10 ml/kg and 14 ml/kg group of big mice, after taking medicine, the body temperature are all lower than that of control group ($P < 0.05$) in the same time points; For the big mice in Aspirin group and bad cold resistance mixture in four time points of 0.5 h, 1 h, 2 h, 3 h, the change situation of big mice temperature is lower than that of control group ($P < 0.05$). For different medicated groups, after 3 hours' medicine taking for the animal models, the content of IL-1α and total white cell number in the peripheral blood has different degree of decrease to some extent by comparing with that of control group, among which, the influence of bad cold resistance mixture 14 ml/kg group on the white cell content in peripheral blood is more obvious ($P < 0.05$). Bad cold resistance mixture 6 ml/kg, 10 ml/kg and 14 ml/kg groups can significantly reduce the content($P > 0.05$) of IL-1α in the peripheral blood of big mice, which has no significant difference ($P > 0.05$) compared with the aspirin and ShuangHuangLian groups. Conclusion: The function of bad cold resistance mixture on the exogenous high fever of animal model can significantly inhibit the increase of body temperature and achieve the goal of antiphlogosis and abatement of fever.

Introduction

Exogenous high fever belongs to clinical common upper respiratory tract infection disease, which has the characteristics of urgnet disease-attack and fast disease progression. The TCM holds the opinion that the reason of exogenous high fever is the disorder of cold and hot and attack of exogenous virus, which is similar to the description of fervescence in modern medicine[1]. The common clinical symptoms of exogenous high fever are fever, pharyngalgia and headache *etc*., and it is also easy to cause lung Qi obstruction and muscle soreness. As the focus of TCM emergency study, adopting the traditional Chinese medicine treatment for external high fever has the distinct advantage. Cold resistance mixture is composed of a variety of new pure traditional Chinese medicine and by combining TCM theory and clinical experience, choose skullcap, radix bupleuri and artemisia apiacea as the main raw materials, which has good curative effect on the exogenous epidemic toxin, besides, it has the effect of abatement of fever and muscle symptoms relieving, regulating organism and pancreas and physiology dispersion. This study, on the basis of

perfecting the extraction process of bad cold resistance mixture and designing bad cold resistance mixture quality control index and through building exogenous high fever animal model, measures the total number and body temperature change of IL-1α and white cell in the peripheral blood of animal model, besides, analyzes the functional mechanism of bad cold resistance mixture so as to provide safer, more reliable and more efficient traditional Chinese medicine (TCM) preparations for clinic.

Materials and Methods

Bad cold resistance mixture: consisting of radix bupleuri, skullcap and plaster stone *etc*., and being made to mixture by related craft. Aspirin enteric-coated tablet(100mg/piece); Shuanghuanglian oral solution. Lipopolysaccharide(10mg/piece), sodium chloride injection(500ml/bottle), 5-HT ELISA *etc*. Main instruments: high-speed refrigerated centrifugal machine, blood routine analyzer, electronic clinical thermometer, homoiothermal water bath, *etc*.

1.2 Experimental Animal

Choose 100 healthy male mice, who all have animal production licenses and their weight are between 180 and 220 grams. Fed them in transparent plastic boxes in animal laboratory, and a box with no more than six, besides, the room temperature is controlled in 20 to 24℃ with humidity of 45% to 70% and illuminance between 150-280 lx. Use standard fodder to feed daily and keep continuous feeding for seven days, besides, according to the real situation, replace the box timely and keep free feeding and water drinking.

After keep continuous feeding of 100 male SD mice for a week, measuring the anus temperature for two times in the daily morning time and the interval time is 1 hour, keeping for 3 days in a row. Choose at least twice of the measurement in the three days and among which, the number of the animals with temperature difference less than 0.5℃ and average body temperature between 36 to 38℃ is 60. Divide the mice equally into six groups, namely groups with bad cold resistance mixture 6 ml/kg, 10 ml/kg 14 ml/kg, control group, aspirin group(100 mg/kg) and ShuangHuangLian group (8 ml/kg) and 10 mice are included n each group.

Use normal to dilute the lipopolysaccharide solution with the concentration control of 0.8 μg/ml and modeling dose control of 0.2 μg/kg. Inject the LPS diluent into the mice through mice enterocoelia to form the exogenous high fever animal model. Generally, after half an hour of modeling, the mice will have the fever reaction along with less exercise, shortness of breath, increased body temperature and food refusal. After modeling, take the body temperature of each mouse for several times within 2h and take one-time dosage when the body temperature reaches up to the peak value, and then take temperature again in 0.5 h, 1 h, 2 h, 3 h, 5 h after the dose. Adopt the cannula way for gavage dosage and control the volume of each one to about 10 ml, besides, for the control group, feed the mice with equal quantity of normal saline instead of medication. In order to reduce the experimental error, all animals are given the the method of parallel operation and given the same thermometer for the measurement [2].

After the building of animal model and the dosage for 3h, take blood specimen collection for each big mouse. The specific operation: choose disposable syringe to take 2 ml of blood from the big mouse’s eyes, and take out about 1.0 ml to lay it in the conventional anticoagulant tube and then place it upside down along with good blending for measurement. Inject the remaining blood slowly into the centrifuge tube and separate the upper serum clear, later according to the reagent operation standard, take the active factor measurement.

Choose SPSS18.0 statistical software to take the data analysis and use X2 to test the enumeration data comparison, besides, for each group of data, use the mean value standard

deviation（ $\bar{x}\pm s$ ） to represent , and the $P < 0.05$ represents that the difference is significant and meaningful.

Results

Compared with the basic body temperature before the building of animal model, the body temperature of big mice in control group after getting the injection of LPS solution for 0.5 h has risen and after 3 h, the temperature reach up to the peak value, later the temperature gradually decreases. Compared with the control group in the same time, different dosage groups, after the building of animal model and dosage, the temperature decreased to some extent, in which, the mice in bad cold resistance mixture 10 ml/kg and 14 ml/kg groups, after dosage, the temperature changes are all lower than that of control group ($P < 0.05$) in the same point; For the mice in Aspirin group and bad cold resistance mixture 6 ml/kg group, in the time of 0.5 h, 1 h, 2 h, 3 h, the change of body temperature is lower than that of control group ($P < 0.05$); The mice in ShuangHuangLian group, in two time points of 1 h, 2 h, have a lower temperature change than that of control group ($P < 0.05$) and the function in other time points have no significant difference ($P > 0.05$). For the above, we can know that the bad cold resistance mixture has significant inhibitory effect on the exogenous high fever of animal models caused by LPS. The specific content is as table 1.

Table 1 Body Temperature Change Condition Comparison of Exogenous High Fever of Animal Models

Groups	Basic body temperature（℃）	Body temperature change values（ $\bar{x}\pm s$，℃ ）				
		0.5h	1h	2h	3h	5h
Control group	37.1±0.58	0.88±0.37	1.12±0.56	1.23±0.62	1.30±0.47	1.03±0.73
Aspirin group	37.2±0.06	0.38±0.56	0.37±0.67	0.62±0.39	0.55±0.24	0.87±0.82
Shuanghuanglian group	37.1±0.62	0.69±0.43	0.59±0.43	0.62±0.33	0.96±0.27	0.92±0.68
Bad cold resistance 6ml group	37.1±0.52	0.42±0.25	0.43±0.15	0.39±0.37	0.32±0.68	0.82±0.57
Bad cold resistance 10ml group	37.2±0.04	0.41±0.16	0.38±0.27	0.36±0.09	0.42±0.25	0.49±0.19
Bad cold resistance 14ml group	37.1±0.49	0.38±0.28	0.36±0.43	0.35±0.62	0.45±0.18	0.52±0.33

After 3 h's dosage for animal models in different groups, the total quantity of IL-1α and white cells in the peripheral blood has different degree of increase to some extent compared with that of control group. Among which, the influence of bad cold resistance mixture 14 ml/kg on the white cells quantity in peripheral blood is more significant ($P < 0.05$), which has significant differences compared with other groups ($P < 0.05$). And the comparison between bad cold resistance mixture 6 ml/kg group and aspirin group, bad cold resistance mixture 6 ml/kg group and ShuangHuangLian group have no significant difference ($P > 0.05$). Bad cold resistance mixture 6 ml/kg, 10 ml/kg and 14 ml/kg groups can significantly reduce the content of IL-1α（$P>0.05$） in mice' peripheral blood,

which has no significant difference (P > 0.05) compared with that of aspirin group and ShuangHuangLian group, in which, the influence on IL-1α of bad cold resistance mixture 14 ml/kg group is the most significant (P > 0.05). So we can know that, the different doses of bad cold resistance mixture have influence on the IL-1α in serum of exogenous high fever of mice, of which, the effect of the highest dose is the best. Aspirin and ShuangHuangLian also have the effect, however, the effect is not good as that of the high dose of bad cold resistance mixture group. The specific data is as the table 2.

Table 2 Situation of serum indicator changes for exogenous high fever of animal models ($\bar{x}\pm s$)

Groups	Animal number	Total number of white cells（10^9/L）	IL-1α（ηg/ml）
Control group	10	14.86±1.423	1.15±0.059
Aspirin group	10	8.57±0.531	0.833±0.064
Shuanghuanglian group	10	8.69±0.362	0.886±0.076
Bad cold resistance 6ml group	10	8.63±0.452	0.831±0.057
Bad cold resistance 10ml group	10	7.53±0.335	0.798±0.046
Bad cold resistance 14ml group	10	6.37±0.628	0.764±0.071

Conclusion

Exogenous high fever belongs to the common emergency of TCM, if it does not get a prompt treatment, it will be easy to lead to many complications [3]. The study of exogenous high fever is closely connected with the body's immune response, especially for the study of antipyretic medicine function mechanism, so it is necessary to build animal models similar to body fever reaction. There is no standard exogenous high fever animal models in the filed of TCM, and the function of LPS on the body can stimulate the temperature-regulating nervus centralis and cause the change of the temperature regulating point and then cause the increase of body's heat production, besides, the LPS intraperitoneal injection is also the classic inflammatory body heating-up method. In the aspect of experimental animals, choose healthy male rice, because its heat reaction is stable, which is easy to monitor [4]. After the animal model was built, the big mice firstly have the reactions of lacking in strength, eating little and tiredness *etc.*, and after about 1 h, the body temperature increases gradually, along with the reactions of shortness of breath and chills *etc*., which is consistent with the clinical symptoms of exogenous high fever. For the mice in the experiment, after the treatment of aspirin, bad cold resistance mixture and ShuangHuangLian mixture, the fever symptom has certain degree of remission compared with the control group, which indicates that the building of animal model is successful .

The body temperature of human and mammal is relatively constant and it is regulated by body temperature center [5]. TCM holds that exogenous high fever is caused by the partial imbalances of internal body and then the external factors get involved, so the both two factors cause the diseases [6]. The reason for it includes internal reason and external reason, of which, the internal reason is

associated with the body constitution attacked by external virus and the internal environment determines the prognoses of body on external virus, besides, the external cause includes dampness and cold *etc.* many factors. The interpretation of exogenous high fever in *Plain Questions* is that 'Overabundance of yang causes hot and overabundance of Yin causes cold' [7]. The fever reaction caused by LPS in this experiment achieves the indirect regulation by external injection of highly active macromolecular substances, which can stimulate the release of body's inflammatory cells and then cause the fever. The study shows that, IL-1α is the important factor for LPS's induction [8]. IL-1α belongs to common endogenous pyrogens and polypeptide caused by the co-function of macrophage and endothelial cells besides, it has significant influence on the temperature center. The function of white blood cells lies in realizing the organism defense and the total number of white blood cells in the peripheral blood of organism is one of the important indicators of infection status.

Bad cold resistance mixture has better curative effect on the long-time high fever and repeated cold and hot caused by common clinical exogenous virus. The characteristics of the treatment of exogenous fever takes the evidence-based medicine as the essential, for which, firstly relieve the exterior syndrome and bring down the fever, and then clear away heat and toxic materials. In summary, the method has the effect of muscle symptoms removing, wind and heat dispersing, lung-diffusing and cough-suppressing. The experiment chooses the LPS to build the animal model of exogenous high fever and takes the treatment in the peak value of fever and then measure the body temperature situation of different time points. Results show that the mice in control group, after getting the injection of LPS for 0.5 h has the phenomenon of body temperature increase, which reached the peak in 3 h, besides, the symptoms of fatigue and chills *etc.* appear. Besides, by comparing with the result of control group, after modeling medication, the body temperature of each treatment group has decreased to some extent. The body temperature of mice in bad cold resistance mixture 10 ml/kg and 14 ml/kg groups, after the treatment, are lower than that of control group in the same time points($P < 0.05$). For the different dosage groups, after 3 h of treatment, the total number of IL-1α and leukocytes in the peripheral blood has different degree of decrease compared with control group. Of which, the influence of bad cold resistance mixture 14 ml/kg group on the number of leukocytes in peripheral blood is more significant ($P < 0.05$). Bad cold resistance mixture 6 ml/kg, 10 ml/kg and 14 ml/kg groups can significantly reduce the content of IL-1α（$P>0.05$） in mice' peripheral blood. It is noted that the bad cold resistance mixture can significantly inhibit the abnormal change of the content of IL-1α and white blood cells in big mice' blood, which can indirectly play the role of regulating temperature center, thus achieve the purpose of reducing inflammation and bringing down fever.

To sum up, bad cold resistance mixture has significant inhibitory effect on the temperature and febrile medium *etc.* exogenous high fever animal model, which has provided theory basis for developing Chinese medicine compound preparations of preventing and curing exogenous high fever. However, due to the exogenous high fever belongs to complex pathological process, it lacks unified index and clinical efficacy evaluation system in the aspect of experiment, so the functional mechanism of bad cold resistance mixture still needs further research.

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