SPANDIDOS PUBLICATIONS

EXPERIMENTAL AND THERAPEUTIC MEDICINE 17: 1545-1550, 2019

Effect of total glucosides of paeony on the changes of IL-4 and ICAM-1 levels in eczema mouse model serum

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Received May 16, 2018; Accepted November 14, 2018

DOI: 10.3892/etm.2018.7122

Abstract. Effect of total glucosides of paeony on the changes of IL-4 and ICAM-1 levels in eczema mouse model serum was investigated. A total of 38 KM mice of SPF grade were divided into 3 groups: the control group (n=10), the model group (n=15) and the treatment group (n=13). The pathological model of chronic eczema in mouse right ear was induced using dinitrochlorobenzene acetone solution. Two ears of mice in the control group and the left ear of mice in the model and treatment groups were smeared with acetone as control. The mice in the treatment group were treated by administration with total glucoside of paeony. The changes of IL-4 and ICAM-1 levels were measured using caudal vein blood collection. The mouse ear weight was measured and the relationship among IL-4 and ICAM-1 levels, ear thickness and treatment time was analyzed. Mouse ear thickness in the model group was higher than that in the treatment and control groups (P<0.05). The weight of the mouse right ear in the model and treatment groups was significantly higher than that of the left ear (P<0.05). Furthermore, The IL-4 and ICAM-1 levels of mice in the model group were higher than that in the treatment and control groups (P<0.05). The IL-4 and ICAM-1 levels of mice in the model and treatment groups increased compared to that before modeling (P<0.05). The IL-4 and ICAM-1 levels of mice were positively correlated with ear thickness in the model group (r=0.865, P=0.002; r=0.833, P=0.009). In addition, the IL-4 level of mice was positively correlated with the ICAM-1 level in the model group (r=0.812, P=0.014). Finally, IL-4 and ICAM-1 may be involved in the pathologic process of chronic eczema. Therefore, the study showed that the total glucosides of paeony may play a role in the treatment of chronic eczema by regulating the IL-4 and ICAM-1 levels.

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Key words: total glucosides of paeony, eczema, mouse model, IL-4, ICAM-1, ear thickness

Introduction

Chronic eczema, which is an allergic inflammatory dermatosis with high incidence in dermatology, belongs to type IV allergic dermatosis, and severe itching, symmetrical location, multiple lesions and recurrent attacks are four main symptoms of eczema (1,2). In recent years, due to global warming, excessive use of chemical industry and food additives, and the acceleration of social development, the incidence of chronic eczema is increasing year by year and is prevalent worldwide (3,4). Many studies on the specific causes of chronic eczema have indicated that immune dysfunction, genetic and environmental factors play an important role (5,6).

Currently, H1 receptor antagonists such as phenhydramine, chlorpheniramine and glucocorticoids are the main drugs for the treatment of chronic eczema, but the clinical staff and patients are not satisfied with the therapeutic effect because of adverse reactions such as dizziness, nausea, lethargy, full moon face and buffalo back, and the application is also greatly limited (7,8). Therefore, it is very important to find new safe and effective drugs. Total glucoside of paeony is the first anti-inflammatory and immunomodulatory drug to be approved for sale and extracted from root of Radix Paeoniae Paeoniae Alba, showing a good prospect in the treatment of inflammatory and immune diseases such as rheumatoid arthritis, effectively improving the immune balance and having good safety (9,10). Inflammatory reactions and immune damage also occurs in the pathogenesis of chronic eczema, including red swelling and allergic reactions (11). Therefore, total glucosides of paeony may also be effective in the treatment of chronic eczema, IL-4 may respond to the immune reaction and ICAM may respond to inflammatory reaction (12,13). We speculate that the regulation of IL-4 and ICAM is one of the mechanisms of the action of total glucoside of paeony.

In this study, a pathological model of KM mice with chronic eczema was established to explore the therapeutic effect and mechanism of total glucosides of paeony in chronic eczema.

Materials and methods

Forty mature KM mice of SPF grade were purchased from the Animal Experimental Center of Gansu College of Traditional Chinese Medicine (Lanzhou, China) [production license SCXK (Gan) 2004-0006-0000911] and all were fed with full

price nutrient pellet feed (Jiangsu Medison Biopharmaceutical Co., Ltd., Jiansgsu, China; website: http://www.medison.co.il/). KM mice were aged 45-55 days with an average age of 47.5±2.5 days and body weight 30-35 g with an average body weight of 33.4±2.7 g. The feeding temperature was 16-26˚C with relative humidity 55.6±8.3% and the mice were kept separately in the feeding box. The nest changed 1-2 times a week, ambient noise was <85 dB, ammonia concentration was not more than 20 ppm with ventilation 8-12 times per hour. The fluorescent lamp interval was a 12 h light/dark cycle. At last, the mice could feed and drink freely, with the feeding box changing 3-4 times a week and the water bottle changing 2-3 times a week. Ten mice were selected as the control group using random number table and the remaining 30 mice were used to establish chronic eczema model and randomly divided into the model and treatment groups after the model was successfully established.

The study was approved by the Ethics Committee of The People's Hospital of Danyang (Zhenjiang, China).

Establishment of rat model. Hair of KM mice was cut off ~3 x 3 cm from the abdomen using electric clipper one day before sensitization, and 5% dinitrochlorobenzene acetone solution (DNCB) (a product of Chemical Reagent Co., Ltd., Shanghai, China) 100 μl was smeared to the abdomen of mice for sensitization on the 1st day of the experiment. On the 6th day, the mice were stimulated with 1% DNCB 5 μl on the medial side of the right ear, while the left ear was treated with acetone once three times for four times.

Treatment methods. After grinding total glucosides of paeony (guo yao zhun zi H20055058; Liwah Pharma, Hangzhou, China; website: http://www.nbliwah.com/) into powder form, the suspension of 10 mg/ml was prepared with 0.5% sodium carboxymethylcellulose, and each medication group was treated with administration of the drug on the same day as sensitization. The treatment group was treated with total glucoside of paeony suspension of 60 mg/kg/day, while the control and non-intervention model groups were given equivoluminal 0.5% sodium carboxymethylcellulose as control until the last stimulation of mice on the 18th day.

ELISA. The peripheral blood of mice was collected using caudal vein blood collection before modeling, at day 1, 6, 9, 12 and 15. IL-4 and ICAM-1 in serum were tested using ELISA. The methods were carried out according to the kit instructions. IL-4 and ICAM-1 test kits were purchased from Shanghai Crystal Pure Reagent Co., Ltd. (Shanghai, China).

Observation indicators. The ear thickness and weight before and after modeling were observed. The thickness difference of the middle right ear of mice was measured using vernier caliper. The rats were sacrificed 24 h after the last stimulation. The same position of the two ears was obtained using 6 mm perforator and the quality of it was measured using electronic balance. The changes of IL-4 and ICAM-1 levels in mouse serum were measured before and after administration of the drug. The correlation of IL-4 and ICAM-1 levels with ear thickness and treatment time was observed.

Statistical analysis. SPSS19.0 [AsiaAnalytics (formerly SPSS China), Shanghai, China] was used for statistical analysis. The enumeration data were expressed by rate and the comparison of rate was tested using χ^2 test. The measurement data were expressed as mean ± standard deviation, the comparison among multiple groups was analyzed using ANOVA, the comparison between two groups was tested using LSD, and repeated variance measurement experiment was used to compare different time within the group. The correlation of IL-4 and ICAM-1 levels with ear thickness and treatment time was analyzed using Spearman's correlation analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Result of modeling. The chronic eczema model was established in 30 KM mice, 28 mice were successfully established, and the success rate of modeling was 93.3%. Two mice in the treatment group died midway because of the drug, but the data were not included in the data of mice in the treatment group. The abdominal texture of mice in the control group was soft, the color was bright red, and there was no red swelling, scab skin and keratosis of the epidermis. After sensitizing mice in the model group, slight red swelling occurred in the abdominal skin, and scratches were seen in individual mouse skin. After the last stimulation, a variety of bright red patches occurred in the abdominal skin of the mice, the texture was relatively hard, and there were also scratches and edema in varying degrees.

Changes of mouse ear thickness. There was no significant difference in ear thickness between the three groups of mice before modeling and at day 1 ($P>0.05$), but there were differences at day 6, 9, 12, 15 and 18 (all $P<0.05$). Mouse ear thickness in the model group was higher than that in the treatment and control groups (all $P<0.05$), and that in the treatment group was higher than that in the control group ($P<0.05$). There was no significant change in mouse ear thickness in the control group during the experiment period ($P>0.05$), and the mouse ear thickness in the model and treatment groups increased compared to that before modeling and continued to significantly increase from day 6 ($P<0.05$) (Table I).

Measurement results of mouse ear weight. There was no significant difference in mouse binaural weight in the control group ($P>0.05$), and the weight of mouse right ear in the model and treatment groups was significantly higher than that of left ear ($P<0.05$). There was no significant difference in the weight of mouse left ear between the three groups ($P>0.05$), but that of right ear was significantly different ($P<0.05$), and the model group was higher than the treatment and control groups (all $P<0.05$), and the treatment group was higher than the control group ($P<0.05$) (Fig. 1).

Changes of IL-4 level in mouse serum. There was no significant difference in IL-4 level between the three groups of mice before modeling ($P>0.05$), but there were significant differences at day 1, 6, 9, 12, 15 and 18 (all $P<0.05$). The IL-4 level of mice in the model group was higher than that in the treatment and control groups (all $P<0.05$), and that in the treatment group was higher than that in the control group (all $P<0.05$).

Table I. Changes of mouse ear thickness (mm).

Variables	Control group	Model group	Treatment group	Statistical value	P-value
Number (a)	10	15	13		
Before modeling	0.173±0.012	0.176±0.017	0.174±0.014	0.135	0.894
day 1	0.177±0.015	0.179±0.018	0.180±0.021	0.076	0.927
day 6	0.183±0.024	0.252±0.032[a,b,g]	0.201±0.022[a,b,h]	22.580	<0.001
day 9	0.186±0.023	0.315±0.035[a-c,g]	0.244±0.027[a-c,h]	58.829	<0.001
day 12	0.192±0.027	0.341±0.041[a-c,g]	0.298±0.031[a-d,h]	56.991	<0.001
day 15	0.202±0.031	0.391±0.046[a-e,g]	0.312±0.032[a-d,h]	74.216	<0.001
day 18	0.221±0.032	0.416±0.043[a-e,g]	0.345±0.032[a-f,h]	84.504	<0.001

[a]P<0.05, compared to before modeling. [b]P<0.05, compared to day 1. [c]P<0.05, compared to day 6. [d]P<0.05, compared to day 9. [e]P<0.05, compared to day 12. [f]P<0.05, compared to day 15. [g]P<0.05, compared to the control group. [h]P<0.05, compared to the model group.

Table II. Changes of IL-4 level in mice serum (ng/ml).

Variables	Control group	Model group	Treatment group	Statistical value	P-value
Number (a)	10	15	13		
Before modeling	0.362±0.032	0.363±0.031	0.362±0.032	0.005	0.996
day 1	0.369±0.031	0.432±0.032[a,g]	0.397±0.033[a,h]	11.958	0.001
day 6	0.365±0.032	0.501±0.035[a,b,g]	0.425±0.032[a,b,h]	52.049	<0.001
day 9	0.364±0.033	0.585±0.036[a-c,g]	0.458±0.034[a-c,h]	64.970	<0.001
day 12	0.367±0.032	0.622±0.039[a-d,g]	0.472±0.035[a-d,h]	70.648	<0.001
day 15	0.362±0.033	0.694±0.041[a-e,g]	0.492±0.037[a-e,h]	76.976	<0.001
day 18	0.366±0.031	0.734±0.052[a-g]	0.512±0.036[a-f,h]	81.631	<0.001

[a]P<0.05, compared to before modeling. [b]P<0.05, compared to day 1. [c]P<0.05, compared to day 6. [d]P<0.05, compared to day 9. [e]P<0.05, compared to day 12. [f]P<0.05, compared to day 15. [g]P<0.05, compared to the control group. [h]P<0.05, compared to the model group.

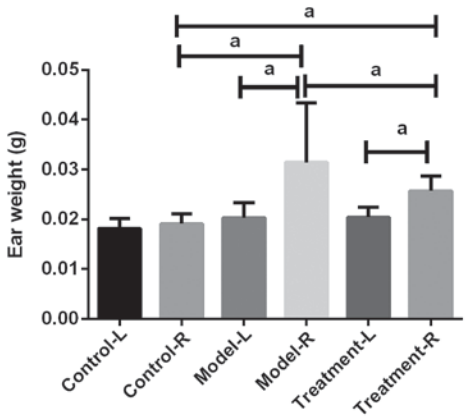

Figure 1. Measurement results of mouse ear weight. There was no significant difference in mouse binaural weight in the control group (P>0.05), and the weight of mouse right ear in the model and treatment groups was significantly higher than that of left ear (P<0.05). There was no significant difference in the weight of mouse left ear between the three groups (P>0.05), but that of right ear was significantly different (P<0.05), and the model group was higher than the treatment and control groups (all P<0.05), and the treatment group was higher than the control group (P<0.05). [a]P<0.05.

There was no significant change in IL-4 level of mice in the control group during the experiment period (P<0.05). Finally, the IL-4 level in mice in the model group increased compared to that before modeling and continued to significantly increase from day 1 (all P<0.05) (Table II).

Changes of ICAM-1 level in mouse serum. There was no significant difference in ICAM-1 level between the three groups of mice before modeling (P>0.05), but there were significant differences at day 1, 6, 9, 12, 15 and 18 (all P<0.05). The ICAM-1 level of mice in the model group was higher than that in the treatment and control groups (all P<0.05), and that in the treatment group was higher than that in the control group (all P<0.05). There was no significant change in ICAM-1 level in mice in the control group during the experiment period (P<0.05). The ICAM-1 level in mice in the model group increased compared to that before modeling and continued to significantly increase from day 1 (P<0.05), the ICAM-1 level in mice in the treatment group increased compared to that before modeling and continued to significantly increase from day 6 (P<0.05) (Table III).

Correlation analysis. The results of Spearman's correlation analysis showed that the IL-4 and ICAM-1 levels in mice were positively correlated with ear thickness in the model group (r=0.865, P=0.002; r=0.833, P=0.009). The IL-4 level in

Table III. Changes of ICAM-1 level in mouse serum (ng/ml).

Variables	Control group	Model group	Treatment group	Statistical value	P-value
Number (a)	10	15	13		
Before modeling	2.25±0.17	2.23±0.18	2.24±0.16	0.042	0.959
day 1	2.27±0.12	2.45±0.19[a,g]	2.33±0.15[h]	4.140	0.024
day 6	2.26±0.14	3.12±0.21[a,b,g]	2.89±0.16[a,b,h]	22.301	<0.001
day 9	2.28±0.15	4.57±0.22[a-c,g]	3.42±0.18[a-c,h]	44.402	<0.001
day 12	2.25±0.17	6.33±0.25[a-d,g]	4.01±0.17[a-d,h]	68.754	<0.001
day 15	2.24±0.16	8.12±0.29[a-e,g]	4.78±0.21[a-e,h]	71.251	<0.001
day 18	2.28±0.18	8.65±0.31[a-g]	5.12±0.19[a-f,h]	82.336	<0.001

[a]P<0.05, compared to before modeling. [b]P<0.05, compared to day 1. [c]P<0.05, compared to day 6. [d]P<0.05, compared to day 9. [e]P<0.05, compared to day 12. [f]P<0.05, compared to day 15. [g]P<0.05, compared to the control group. [h]P<0.05, compared to the model group.

Table IV. Correlation analysis.

Groups	Ear thickness	IL-4	Treatment time
Model group			
IL-4	r=0.865		
	P=0.002		
ICAM-1	r=0.833	r=0.812	
	P=0.009	P=0.014	
Treatment group			
IL-4			r=0.712
			P=0.024
ICAM-1			r=0.734
			P=0.021

mice was positively correlated with and the ICAM-1 level in the model group (r=0.812, P=0.014). IL-4 and ICAM-1 levels in mice were positively correlated with the treatment time in the treatment group (r=0.712, P=0.024; r=0.734, P=0.021) (Table IV).

Discussion

Chronic eczema is a global public health problem (14), and the immune imbalance of Th2/Th1 plays a crucial role in the occurrence and development of chronic eczema, contributing to abnormal secretion of related cytokines such as IL-4 and ICAM-1 (15,16). Mouse IL-4, which is mainly produced by Th2 subgroup and participates in humoral immunity, is also an important indicator reflecting the degree of immune reaction of Th2 and Th1 cells (12) ICAM-1, which belongs to the immunoglobulin superfamily of adhesion molecules, is an important adhesion molecule in mediating adhesion reaction, playing an important role in the promotion of the adhesion of inflammatory sites and the regulation of the immune reaction of the body (13). Total glucoside of paeony is an anti-inflammatory and immunomodulatory drug (9), but there are few reports on the related treatment of chronic eczema, and the mechanism is not clear. In this study, we explored the curative effect and the mechanism of total glucosides of paeony through dynamically monitoring the changes of IL-4 and ICAM-1 levels in mice with chronic eczema during the treatment with total glucoside of paeony.

In this study, DNCB was used to sensitize mice to induce inflammatory reaction and to replicate the pathological model of chronic eczema, which is a classic animal model (17). This study showed that the mice developed related pathological manifestations such as red swelling, plaque and scratches after the last stimulation of DNCB, suggesting that the model was successfully established, similar to the manifestations of mice model with chronic eczema in related reports (18,19). However, two mice in the treatment group died in this experiment. The maximum dose of administration was 0.21 ml in this experiment, while the maximum dose of tolerance of mice was 0.5 ml (20), so we speculated that the death of mice might be caused by the drug. In this study, from the measurement results of mouse ear thickness, it could be seen that with the increase of stimulation times, the mouse ear thickness in the model and treatment groups increased, but that of the treatment group was significantly lower than that of the model group. The curative effect of anti-dermatitis drug could be judged according to the degree of ear swelling (21). The results of this study also showed that the mouse ear quality in the treatment group was lower than that in the model group, suggesting that total glucoside of paeony can obviously improve the mouse ear thickness and quality. It indicated that total glucosides of paeony had a good therapeutic effect on mice with chronic eczema, which might be related to the improvement of mice with inflammatory and immune injury with total glucosides of paeony. In this study, the changes of IL-4 and ICAM-1 levels were dynamically monitored during the sensitization and stimulation of mice, and the IL-4 and ICAM-1 levels in mice in the model group also increased with the increase of stimulation times. Spearman's correlation analysis also showed that IL-4 and ICAM-1 levels were positively correlated with ear thickness. Song *et al* (22) showed that the IL-4 level in eczema patients increased significantly, and it was higher as the severity of eczema increased. Studies reported by them indicated that the IL-4 level in eczema patient serum increased significantly (23,24). We speculated that this may be related to the immune imbalance of Th2/Th1 in mice with chronic

eczema, and there was an immune imbalance of Th2/Th1 in chronic eczema mice, which was dominated by the activation of Th2 cells, thus leading to the increase of IL-4 expression level in mice (25,26). Huang *et al* (27) and Han *et al* (28) also reported that ICAM-1 level in chronic eczema patients were significantly higher than those in normal controls, similar to our results. ICAM-1 can be expressed in keratinocytes under the stimulation of sensitization, while scales, as one of the special diagnosis of chronic eczema, can further develop keratinocytes (29,30), which may also be the reason for the increase of ICAM-1 level in chronic eczema patients. Although the IL-4 and ICAM-1 levels in mice in the treatment group also increased with the increase of stimulation times, and Spearman's correlation analysis also showed that the IL-4 and ICAM-1 levels in mice in the treatment group were positively correlated with the treatment time and the level in the treatment group was significantly lower than that in the model group, suggesting that total glucosides of paeony may improve the inflammatory immune injury of mice by improving IL-4 and ICAM-1 levels. In recent years, there have been few reports on the treatment of chronic eczema with total glucosides of paeony. In the study of Wang *et al* (9), it was indicated that total glucosides of paeony could improve the ICAM-1 level in mice with allergic contact dermatitis. In a previous study, it was shown that total glucosides of paeony may improve the ICAM-1 level in diabetic rats (31). Both allergic contact dermatitis and diabetes are immune diseases, so we can believe that total glucosides of paeony have a function in the improvement of ICAM-1 level in chronic eczema mice. However, mice and humans have more similarities, but the conclusion cannot represent the results of clinical trials, so we still need more clinical data to prove our conclusions.

In conclusion, IL-4 and ICAM-1 may be involved in the pathologic process of chronic eczema and total glucosides of paeony may play a role in the treatment of chronic eczema by regulating the IL-4 and ICAM-1 levels.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

MZ drafted the manuscript. HS established the rat model. MZ and HS were responsible for ELISA and observation indicators. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The People's Hospital of Danyang (Zhenjiang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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