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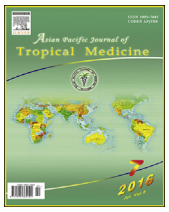

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Study on the therapeutic mechanisms of pseudolaric acid in mice with allergic contact dermatitis

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ABSTRACT

Objective: To study the therapeutic mechanisms of pseudolaric acid on allergic contact dermatitis in mice.

Methods: A total of 50 BALB/C mice were selected and randomly divided into control group, model group, and treatment A, B, C groups with 10 rats in each group. ACD model was established in model group, and treatment A, B, C groups but not in control group. Model group received no treatment, but treatment A, B, C groups were treated with external application of the concentration of 0.1%, 0.2% and 0.4% of the pseudolaric acid for the lesions of ear skin. And the weight gain and the swelling degree of the mice' ear were recorded, weight of thymus and spleen were measured. Spleen suspension was prepared to test T lymphocyte and B lymphocyte levels of mice in five groups. Changes in serum IFN-γ, IL-4 and IL-10 levels were tested through the enzyme linked immunosorbent assay (ELISA).

Results: The weight gain of mice in model group were significant lower than those of mice in the control group and the treatment A, B, C groups ($P < 0.05$). Weight gain of mice in treatment A, B groups were significant lower than that of control group ($P < 0.05$), but the difference in weight gain between treatment C group and control group showed no significant difference ($P > 0.05$). The swelling degree and the weight of mice ears in model group were significant higher than those of mice in control group and treatment A, B, C groups ($P < 0.05$). Swelling degree and the weight of mice ears of treatment A, B, C groups were obviously higher than that of control group ($P < 0.05$). The swelling degree and weight of mice' ears in treatment A, B, C groups were decreased with the increase of the drug dosage, but comparison between A, B and C group showed statistically differences ($P < 0.05$). The thymus and spleen index of mice in model group were significant higher than those of the other four groups ($P < 0.05$), among the four groups, thymus and spleen index of treatment A and B group were higher than control group and treatment C group ($P < 0.05$). The stimulation index of T and B cells of mice in model group was significantly higher than the rest four groups ($P < 0.05$). The serum IFN-γ level of mice in control group and treatment A, B and C group was obviously lower than that of mice in model group ($P < 0.05$). The serum IFN-γ level of mice in treatment A, B and C group were decreased with the increasing of the drug dosage, and the level of C group was obviously lower than that of A and B group ($P < 0.05$).

Conclusion: The pseudolaric acid has anti-inflammation and immune adjustment the effects showing a remarkable therapeutic effects for the ACD mice.

1. Introduction

Allergic contact dermatitis (ACD) is the skin inflammatory disease caused by skin exposure with allergic source of external environmental, which presents as the symptoms of erythema, papula, edema, blister and even necrosis with different degree of ache, pruritus or burning sensation [1–3]. The allergic contact dermatitis can be induced by many different sensitizers, but most of them are the chemicals of low molecular weight, which can form the antigenic substance of high molecular weight by combined with the epidermal protein [4]. A period

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of 4–14 days after the patients contact with the allergic source is called latent period, and the organism of patients is in the allergic state during this period, and the patients will suffer the ACD within 48 h if they get in touch with the allergic source again [5]. Studies have shown that [6] ACD is the allergic reaction mediated by T-cell, the immune cells and the cytokines, chemotactic factors and inflammatory mediators that secreted by immune cells have the key effect on the occurrence of ACD. In the clinical treatment, the patients should be initially kept away from the allergic source, and treated with the antihistamine drugs, non-steroidal anti-inflammatory drugs and the hormone drugs. However, sometimes it is impossible to avoid the allergic sources completely, and the antihistamine drugs and the hormone drugs could not cure ACD, hence the ACD could severely influence patients' life quality [7]. Pseudolaric acid is the new diterpenoid acid chemical compounds extracted from dry bark of pseudolarix raempferi gordon, which can be used to treat the tinea, and has the effect of insecticidal and anti-itch effects [8]. A research has confirmed that [9] pseudolaric acid has the prominent effects on ACD. The BALB/C mice were selected to establish the ACD model in this study in order to observe the immune adjustment and the treatment effect of the pseudolaric acid on the ACD mice, and the intervention treatment of pseudolaric acid was given to observe its treatment effects and mechanism of action on the ACD mice, which aimed at providing the theoretical basis for the clinical application.

2. Materials and methods

2.1. Experimental animal

A total of 50 SPF grade, BALB/C male mice with ages of 12 weeks and body weight of 18–22 g were selected for the experiment and the mice were provided by the Laboratory Animal Centre of our hospital. The mice were feed freely in the room temperature of (23 ± 3)°C. In the process of the experiment, the handling of animals was strictly abided by the Regulation of Experimental Animals, and approved Ethics Committee of Henan University. This experiment was operated and finished at the Experiment Center of Henan University.

2.2. Medicines and instruments

The pseudolaric acid was purchased from Chengdu Biopurify Phytochemicals Ltd., which was made by emulsifiable paste to the ointment of the depthness of 0.1%, 0.2% and 0.4%. The dinitrofluorobenzene (DNFB) was purchased from Shanghai Shifeng Biological Technology Co., Ltd. The Compound Dexamethasone Acetate Cream was produced by Sanjiu Pharmaceutical Co., Ltd., the dexamethasone sodium phosphate injection was produced by Tianjin Jinyao Amino Acid Co., Ltd., and the ciclosporin was purchased by North China Pharmaceutical Group Corporation Veterinary Co., Ltd. The TS100 type inverted microscope was purchased by Ni Kon Ltd., the biological spectrophotometric meter was purchased by Germany Eppendorf Ltd., the MCO-AC carbon dioxide cell incubator was manufactured by SANYO Electrical Co., Ltd., and the C-4040ZOOM optical microscope was manufactured by Shanghai Ailang Instrument Co., Ltd., the ELISA reader was manufactured by BIO-RAD Co., Ltd.

2.3. Model establishing method

The dinitrofluorobenzene was used to establish the ACD mice model. Method: the 8% of sodium sulfide was used to remove the fur of the fixed position of the rat's abdomen1 day before establishing the model, which was (2 × 20) cm. And the 1% of 30 μL dinitrofluorobenzene was applied on the fur removal part on the first and the second day of establishing model. The fur of the mice back was removed on the sixth day of establishing model, and 5% of 30 μL dinitrofluorobenzene was applied for stimulation, then 24 h later the model was prepared successfully.

2.4. Grouping and treatment

A total of 50 BALB/C mice were randomly divided into control group, model group, treatment A, B and C group with 10 rats in each group. ACD model in the rest 4 groups was induced by the dinitrochlorobenzene (DNCB), but model was not established in control group. Model group received no treatment, the treatment A, B and C group were treated with external application of the concentration of 0.1%, 0.2% and 0.4% of the pseudolaric acid for the lesions of ear skin for twice a day.

2.5. Observational index

The weight gain and the swelling degree of the mice' ear were recorded after the first stimulating, and then the mice were executed at the 8th day of establishing model to test the weight of mice' ears, then the weight of thymus and spleen was measured and their data indexes were calculated. And the spleen suspension was prepared to test T lymphocyte and B lymphocyte levels of mice in five groups. Then the ophthalmic venous plexus blood of mice in five groups was extracted, and the changes of the serum IFN-γ, IL-4 and IL-10 levels were tested through the enzyme linked immunosorbent assay (ELISA).

2.6. Statistical analysis

The SPSS 13.0 software was used for data processing, the experimental data was expressed as mean ± SD, and the one-way analysis of variance was used for the comparison among groups, $P < 0.05$ was statistically different.

3. Results

3.1. Comparison of weight gain, swelling degree of ear and weight of ear of mice among five groups

The weight gain of mice in model group was significant lower than that of mice in control group and treatment A, B and C group ($P < 0.05$), and weight gain of mice in treatment A and B group were significant lower than that in the control group ($P < 0.05$). The comparison of weight gain of mice in treatment C group and control group showed no significant differences ($P > 0.05$). The swelling degree of ear and weight of ear of mice in model group were significant higher than those of mice in control group and treatment A, B and C group ($P < 0.05$), and the swelling degree of ear and weight of ear of treatment A, B and C group were significant higher than that of the control group ($P < 0.05$). The swelling degree of ear and weight of ear