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Study on the effect of different doses of Ermiaosan on inflammatory response in CIA model rats

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Abstract: Objective: To investigate the effect of different doses of Ermiaosan on improving the inflammatory response in collagen-induced arthritis (CIA) model rats. Methods: The CIA rat model was established and randomly divided into the model group (CIA group), and low, medium, and high dose Ermiaosan groups. Each group was treated with the corresponding drugs, while the healthy control group and the model group were given an equal volume of saline by gavage. The expression of inflammatory factors IL-1β, IL-6, and TNF-α in each group was detected using ELISA, and the pathological changes in rat joints were observed using Masson staining. Results: Rats in the CIA group showed significant tissue damage, tissue proliferation, and inflammatory infiltration in the joints. The low, medium, and high dose Ermiaosan groups exhibited reduced joint tissue destruction and proliferation. Different doses of Ermiaosan were able to alleviate joint lesions in CIA rats. The expression levels of IL-1β, IL-6, and TNF-α in serum were significantly reduced in all dose groups ($P<0.05$). Compared with the CIA group, the medium and high dose groups showed consistent therapeutic effects, both significantly better than the low dose group. Conclusion: The medium dose of Ermiaosan achieved satisfactory therapeutic effects on the inflammatory response in CIA model rats. Ermiaosan may exert its therapeutic effects on RA by inhibiting inflammatory factors.

Keywords: Ermiaosan; Rheumatoid Arthritis; CIA Model Rats

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by persistent synovitis and progressive bone destruction in multiple joints. Common symptoms include joint swelling, pain, stiffness, deformity, and severe functional impairment. However, the pathogenesis of RA remains unclear[1]. According to incomplete statistics, the global incidence rate is 0.1-1% of the population, with the incidence in China ranging from 0.32% to 0.36%[2]. Due to the difficulty in controlling its pathological process, high disability rate, and significant impact on patients' physical and mental health and quality of life, RA has become one of the challenging diseases in the medical field. Ermiaosan, composed of Atractylodes lancea and Phellodendron amurense, was first recorded as Cangzhu Powder in "Shiyi Dexiao Fang" by Wei Yilin in the Yuan Dynasty and later renamed Ermiaosan in "Danxi Xinfa" by Zhu Zhenheng. Ermiaosan has the effect of clearing heat and drying dampness, primarily treating damp-heat syndrome, muscle and bone pain, and weakness of the legs and knees, as well as red, swollen, and painful feet and knees. It is a classic prescription for treating damp-heat syndrome. Modern pharmacology has confirmed that Ermiaosan can effectively treat rheumatoid arthritis and acute gout[3-5]. According to the dosage instructions for Ermiaowan, the recommended dosage is 6-9g per dose, taken twice daily. In contrast, "Danxi Xinfa" prescribes 3-5g per dose, taken three times daily. Given the same disease and medication, the dosage recommendations differ. This study aims to explore the optimal dosage by investigating the effects of different doses of Ermiaosan on improving the inflammatory response in collagen-induced arthritis (CIA) model rats.

2. Materials and methods

2.1 Animals

Forty male DBA/1 mice (SPF grade, weight 18-20g) were purchased from Shanghai Slack Laboratory Animal Co., Ltd.

2.2 Preparation of Ermiaosan decoction

For the preparation of Ermiaosan decoction, 100g of Phellodendron amurense and 100g of Atractylodes lancea were soaked in 2000ml of distilled water and boiled for 1 hour. The mixture was filtered and reserved. The residue was then boiled again in 1000ml of distilled water

for 30 minutes, and the filtrates from both boils were combined. The combined filtrate was then heated and concentrated to a final concentration of 1g·ml-1 for use.

3. Methods

3.1 Animal model, grouping, and sample collection

Forty Wistar female rats were used. Ten rats were assigned to the healthy control group, while the remaining thirty rats were subjected to CIA modeling. The initial immunization involved subcutaneous injections at multiple sites with bovine type II collagen at a concentration of 1mg/ml. One week later, a booster immunization was performed with intraperitoneal injections of bovine type II collagen at a concentration of 1mg/ml (0.3ml per rat). The healthy control group received an equal volume of saline subcutaneously at multiple sites during the initial immunization and intraperitoneally one week later.

After successful modeling, the rats were randomly divided into five groups using a random number method: CIA model group (CIA), low-dose Ermiaosan group (1ml of Ermiaosan decoction), medium-dose Ermiaosan group (2ml of Ermiaosan decoction), and high-dose Ermiaosan group (3ml of Ermiaosan decoction). The healthy control and CIA model groups received saline gavage. All treatments were administered daily for 4 weeks.

3.2 Detection of inflammatory cytokines in rat serum by elisa

ELISA was performed according to the kit's instructions. Blank control wells, standard wells, and sample wells were set up, with each test containing three replicate wells and 100μl of sample per well. After gentle shaking, 50μl of enzyme-labeled solution was added to each well, and the plate was incubated at 37°C for 1 hour. The liquid was discarded, and the wells were washed five times. After drying, 50μl each of DAB coloring solutions A and B were added to each well and incubated at 37°C in the dark for 10-15 minutes. Then, 50μl of stop solution was added to each well. Absorbance (A) at 450nm was measured. The experiment was repeated three times.

3.3 Masson staining to observe pathological changes in rat joints

The skin and muscle tissue attached to the surface of the hind joints of the rats in each experimental group were trimmed and decalcified using EDTA. The tissues were then embedded in paraffin and sectioned. The sections were stained with hematoxylin for 10 minutes, rinsed for 1 minute, and then allowed to stand for 5 minutes. They were stained with Ponceau S for 7 minutes, rinsed with 2% acetic acid solution for 5 seconds, differentiated with 1% phosphomolybdic acid solution for 10 minutes, and stained with aniline blue solution for 5 minutes. Finally, the sections were rinsed with 2% acetic acid solution for 5 seconds and observed under a light microscope.

3.4 Statistical Analysis

Experimental data were analyzed using SPSS 24.0 software. One-way ANOVA followed by pairwise comparisons (Tukey test) was performed to analyze differences between groups. Results were expressed as mean ± standard deviation ($\bar{x} \pm s$), and a P value <0.05 was considered statistically significant.

4. Result

4.1 Impact of Ermiaosan on joint pathological features in different experimental groups

Masson staining results showed that, compared to the healthy group, the CIA group exhibited significant inflammatory infiltration and characteristic arthritic changes such as tissue damage and proliferation in the joints. In the different dosage groups of EMS, joint destruction and tissue proliferation were reduced. The middle and high dosage groups showed more noticeable therapeutic effects on arthritis features and tissue proliferation compared to the low dosage group. This indicates that the CIA rat model was successfully established in this experiment, and the optimal therapeutic concentration of EMS for treating CIA rats is the medium dosage.

4.2 Impact of Ermiaosan on the expression of inflammatory cytokines in different experimental groups

The differences in the levels of inflammatory cytokines in the serum of rats from different experimental groups were statistically significant ($P<0.01$). Compared to the healthy group, the levels of IL-1β, IL-6, and TNF-α in the serum of the CIA group were significantly elevated ($P<0.01$). Compared to the CIA group, the expression of IL-1β, IL-6, and TNF-α in the serum of rats from the different dosage groups was significantly reduced ($P<0.05$). The levels of IL-1β and TNF-α in the low dosage group were significantly lower than those in the middle and high dosage groups ($P<0.05$, $P<0.01$), while the difference in IL-6 levels was not statistically significant ($P>0.05$).

Conclusion

RA is a disease for which traditional Chinese medicine offers significant therapeutic advantages, and Ermiaosan has been widely used in its clinical treatment. The CIA rat model is a classic and currently recognized as one of the best models for studying RA, especially in research related to treatment mechanisms and immune responses[6-7]. This study confirms that Ermiaosan at various doses can alleviate joint lesions in CIA rats. Compared to the CIA group, both the medium and high-dose groups demonstrated consistent therapeutic effects, with more pronounced results than the low-dose group. The levels of inflammatory factors IL-1β, IL-6, and TNF-α in the rats' serum were significantly reduced, suggesting that Ermiaosan may exert its therapeutic effects on RA by inhibiting the expression of these inflammatory factors.

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