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Regulation of Janus Kinase-signal Transducers and Activators of Transcription Pathways in Psoriatic Mice by Chinese Herbal Compound and Efficacy of Chinese Traditional Medicine under Nano-suspension Technology

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ABSTRACT

The mechanism of the treatment of psoriasis-like mice with the Chinese herbal compound YinXie No.1 prepared by nano-suspension technology was investigated based on Janus kinase-signal transducers and activators of transcription (JAKs/STATs) pathway. The high-pressure homogenization technology was used in the preparation of the YinXie No.1 nano-suspensions. Then, 50 Kunming mice were equally classified into the negative control group (NC), the psoriasis model group (PsM) prepared with 5% imiquimod cream on the back, the Tripterygium glycosides-gavage group (TrG), the YinXie No.1-gavage group (YX1), and the YinXie No.1 nano-suspension group (Nano-YX1). The pathological changes and the differential expressions of STAT3 and STAT5 were compared in each group after the treatment. The results showed that the particle size of nano-suspension powder was smaller and had strong stability compared with the active pharmaceutical ingredient (API). Compared with the NC group, psoriasis-like lesions were observed in the PsM group. Compared with the PsM group, the conditions of the erythema on skin lesions, the mRNA expression of STAT3 and STAT5, and protein expression of p-STAT3 and p-STAT5 in the TrG group, YX1 group, and Nano-YX1 group were notably decreased (P<0.05). Compared with the TrG group and YX1 group, the improvement effect of various indexes in the Nano-YX1 group was closer to that in the NC group, but there were differences between the NC group (P<0.05). Chinese herbal compound was helpful to regulate and control the JAKs/STATs pathway to improve the symptoms of psoriasis mice, and the preparation of Chinese herbal compound decoction by nano-suspension technology could improve the therapeutic effect.

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Introduction

Psoriasis is a very common skin disease, and the main clinical manifestations are red papules or patches covered with multiple layers of silver-white scales (1). There are nearly 100,000 new cases of psoriasis in China every year. The disease has seriously affected patients' quality of life (2). Genetic factors, lifestyle, and infection can induce or aggravate psoriasis, and autoimmune inflammation and neovascularization are the pathological basis of psoriasis (3). Psoriasis is a polygenic hereditary disease. Several genes, such as human leukocyte antigen (HLA), p63, and interleukin-4 (IL-4), have been proved to be closely related to the progression of psoriasis (4-6). The Janus kinase-signal transducers and activators of transcription (JAKs/STATs) pathway is mainly involved in the regulation of the biological functions of cytokines, which can affect the transcriptional regulation of genes and the differentiation of immune cells, mediate the inflammatory response, and regulate the immune response (7).

Western medicine's clinical treatment for psoriasis is mainly the internal medicines and the topical therapy, which can be classified into anti-tumor drugs, immunosuppressants, biological agents, antibiotics, and corticosteroids (8). Psoriasis is also known as "BaiBi", "chronic eczema", or "Song Pi Xuan" in

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traditional Chinese medicine. Blood heat is the core of the pathogenesis of psoriasis vulgaris, and it is also the basis of the occurrence of this disease (9). Blood heat with wet poison overflows the skin which can cause the disease. It becomes poisonous over time, blood stasis occurs when blood is heated and qi and blood flow is blocked. Consequently, the key to treating this disease is removing pathogenic heat from blood to detoxify and promote blood circulation to remove blood stasis. Chinese traditional medicine has been proved to be effective and safe in the treatment of psoriasis (10). Some drug components have been confirmed to be relatively unstable and difficult to dissolve in water, thus causing the problems of a short half-life period and poor bioavailability (11). Reducing the drug's particle size can increase its solubility and the contact time between the drug and digestive tract to ultimately improve drug bioavailability (12). Nano-suspension is dispersing the drug particles in the solution by using the stabilizing effect of surfactant, and the stable nano colloidal dispersion is prepared by means of grinding (13). At present, there is the dispersive method such as high-pressure homogenization (HPH) and the coagulating method such as the emulsification method, both of which are widely used in the preparation of nano-suspension (14).

The Chinese herbal compound nano-suspension for the treatment of psoriasis was prepared by the HPH method, and the psoriasis-like mouse model was established to explore the therapeutic effect of different treatment methods on psoriasis and the effect of the JAKs/STATs pathway. It aimed to improve the clinical treatment effect of psoriasis and provide experimental materials for the improvement of patients' prognoses.

Materials and methods

The experimental materials

Kunming mice were purchased from Liaoning Changsheng Biotechnology Co., Ltd.Trizol reagent and bicinchoninic acid (BCA) kit were purchased from MerckSigma-Aldrich LLC. TB Green® Premix Ex Taq™ II (Tli RNaseH Plus), Bulk and rimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) were purchased from Takara Biomedical Technology (Tokyo) Co., Ltd. Rabbit polyclonal STAT3 Antibody, Mouse monoclonal STAT5 Antibody, Rabbit polyclonal STAT3 (phosphorylated), Mouse monoclonal STAT5 (phosphorylated), Rabbit polyclonal β-actin antibody, and Rabbit Anti-mouse immunoglobulin G (IgG) H&L hypothalamic regulatory peptides (HRP) were bought from Abcam Ltd. Tripterygium glycosides were bought from Guizhou Hanfang Pharmaceutical Co., Ltd. (national medicine permission number: Z52020369, drug specification: 10mg*100 tablets). The Chinese herbal compound YinXie No.1 was offered by the department of preparation of the Hospital.

The experimental methods

Preparation of Chinese herbal compound nano-suspension agent

The YinXie No.1 nano-suspension for treatment of psoriasis was prepared. 1g medicine was taken according to the formula, and distilled water containing Poloxam P407 was added at a ratio of 1:200 and placed in a homogenizer for high-speed shear treatment at 16,000rpm for 5 min. Subsequently, the suspension was placed in a high-pressure homogenizer and homogenized 5 times at a pressure of 500bar, followed by another 10 times at a pressure of 1,200bar. Finally, the Chinese herbal compound nano-suspension was obtained.

Characteristics analysis of nano-suspension

For particle size distribution, the average particle size and distribution of powders were detected by the particle size distributor.

For particle morphology, the surface morphology of the powders was observed under the scanning electron microscope. Then, the images were collected.

For sedimentation volume ratio, the prepared nano-suspension was placed in the measuring cylinder; and after it was mixed up, the height was measured and recorded (H0). When there was no change in the liquid level, the final height was recorded (H1). The equation of H1/H0 was used to calculate the sedimentation volume ratio, and the sedimentation curve was drawn.

Preparation of animal model of psoriasis and the treatment grouping conditions

50 mice were randomly divided into 5 groups with 10 mice in each group, which were the negative

control group (NC), the psoriasis model group (PsM), the Tripterygium glycosides treatment group (TrG), the YinXie No.1 treatment group (YX1), and the YinXie No.1 nano-suspension treatment group (Nano-YX1). All the experimental animals were given 80mg/kg pentobarbital sodium anesthetics before the experiment, and they were reared in a single cage after their back hair was removed. Mice in the NC group were coated with Vaseline on their back and given a gavage of 0.5mL/d distilled water. Mice in the PsM group were coated with 25mg 5% imiquimod cream on the back and given a gavage of 0.5mL/d distilled water. Mice in the TrG group were coated with 25mg 5% imiquimod cream on the back, and they were given a gavage of 1.5mg/kg/d Tripterygium glycosides tablets. Mice in the YX1 group were coated with 25mg 5% imiquimod cream on the back and given a gavage of 50g/kg/d YinXie No.1 decoction. Mice in the Nano-YX1 group were coated with 25mg 5% imiquimod cream on the back and given a gavage of 50g/kg/d Chinese traditional medicine YinXie No.1 nano-suspension. All the mice were administered twice a day for 7 days.

Grading of lesion area and severity

The changes in skin lesions in each group were observed before and after treatment. Psoriasis area and severity index (PASI) was used to score the degree of erythema, scale, and infiltration (15). The total score was used to evaluate the changes in skin lesions in each group.

Histopathological examination of skin lesions

The mouse skin tissue was fixed with 10% formaldehyde solution and treated with paraffin-embedded. The paraffin section with a thickness of 3μm was prepared by a slicing machine. After the slice was dewaxed, the tissues were stained with hematoxylin-eosin solution, and the histological changes of skin lesions were observed under a microscope (16). The degree of skin thickening was analyzed and recorded.

Detection of real-time fluorescence quantification polymerase chain reaction (RT-qPCR)

The total ribonucleic acid (RNA) was extracted from mouse skin tissue by the Trizol method. After RNA purity and concentration were detected, the expression level of target genes STAT3 (GeneID: NM_011486.5), STAT5 (GeneID: NM_001164062.1) and glyceraldehyde phosphate dehydrogenase (GAPDH) gene (GeneID: Nm_001289726.1) were detected according to the RT-qPCR kit. 2μL complementary deoxyribonucleic acid (cDNA) template, 12.5μL TB Green Premix Ex Taq II (2X), 2μL upstream and downstream primers (1μL each), and 8.5μL ddH_2O were added into the test tube. The mixed reaction system with a final volume of 25μL was prepared. The system was placed into the Applied Biosystems RT-qPCR instrument. The program was set, and the expression level of the target gene was detected. STAT-3, STAT-5, and GAPDH were designed and synthesized by the Sangon Biotech (Shanghai) Co., Ltd. Table 1 showed the information of primer. The GAPDH gene was used as the internal reference, and the relative expression levels of STAT3 and STAT5 were detected by $2^{-\Delta\Delta ct}$ method.

Table 1. Information on RT-qPCR primers

Gene	Primer sequences (5'→3')	Product size
STAT3	F: AGAACCTCCAGGACGACTTTG R: TCACAATGCTTCTCCGCATCT	159
STAT5	F: CGATGCCCTTCACCAGATG R: AGCTGGGTGGCCTTAATGTTC	144
GAPDH	F: TGGATTTGGACGCATTGGTC R: TTTGCACTGGTACGTGTTGAT	211

Western blot detection

The skin lesions of mice were cut into pieces with ophthalmic scissors and homogenized thoroughly in a homogenizer with 500μL tissue lysate. After they were cracked for 30 min, they were placed in an ultra-high-speed and low-temperature centrifuge and centrifuged at 12,000rpm at 4°C for 10 min. The supernatant was taken, and the protein concentration of the sample was detected according to the BCA kit. After the standard curve was drawn, the protein concentration was adjusted. The separation gel and spacer gel with appropriate concentrations were prepared, and the electrophoresis was performed after the sample was loaded. The target protein was transferred to a polyvinylidene fluoride (PVDF) membrane and placed in a blocking solution with 5% bovine serum albumin. Then, they were placed on the table concentrator at room temperature for 1 hour.

After STAT3 (1:1,000), STAT5 (1:1,000), p-STAT3 (1:1,000), p-STAT5 (1:1,000), β-actin (1:5,000) econazole were added, they were incubated overnight in a 4°C refrigerator. The HRP-labeled IgG secondary antibody (1:10,000) was added and incubated at room temperature under dark conditions for 1 hour. The gel imaging system was used for protein development, and Image J was employed for quantitative analysis of the relative gray value of the target protein. The β-actin was used as an internal reference, and the relative expression level of the target protein was calculated.

Statistical treatment

SPSS 19.0 was employed for data statistics and analysis. Mean ± standard deviation ($\bar{x}\pm s$) was how the experimental data were expressed. Results of each group were pairwise compared by using a one-way analysis of variance (ANOVA). The difference was statistically significant with $P<0.05$.

Results and discussion

Quality evaluation of Chinese herbal compound nano-suspension

Firstly, the morphology of the drug was observed by scanning electron microscope, and the average particle size of the drug was evaluated. In Figure 1A, the main shape of the Chinese herbal compound active pharmaceutical ingredient (API) was an irregular square cylinder, and the particle size distribution was in the range of 10μm-150μm with the maldistribution. In Figure 1B, after the preparation of nano-suspension, the main shape of the nano-drug was the regular rice-grain or shuttle shape, and the drug particle size distribution was in the range of 1μm-2μm with relatively uniform distribution. In Figure 1C, the average particle size of the prepared nano-suspension was only 1.7±0.6μm, and the average particle size of the API was 131.6±20.1μm.

The sedimentation curve was drawn to evaluate the drug sedimentation volume ratio. The settlement volume ratio of API suspension and crude drug suspension (coarse-grained after the drug was ground) decreased gradually with the increase of time. The sedimentation volume ratio of nano-suspension had little change at different times (Figure 2). 384 hours later, the settling volume ratio of the API suspension was 0.08, of the crude drug suspension was 0.25, and of the nano-suspension was 0.96.

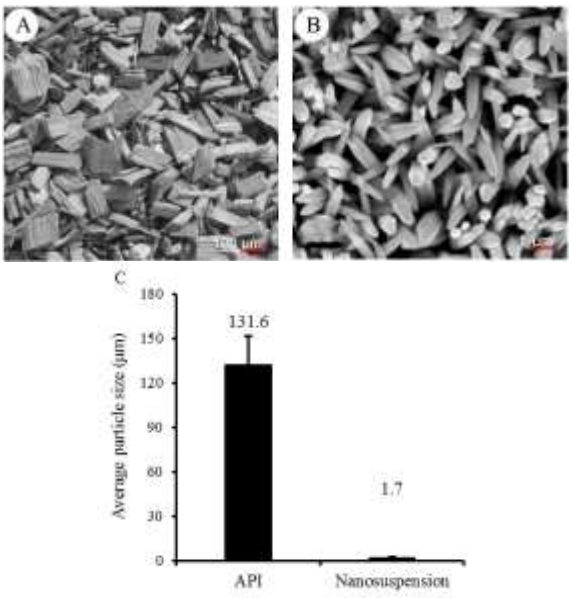

Figure 1. Scanning electron micrographs of drugs and average particle size. (A: active pharmaceutical ingredient; B: nano-suspension powder after the desiccation; C: average particle size; API referred the active pharmaceutical ingredient).

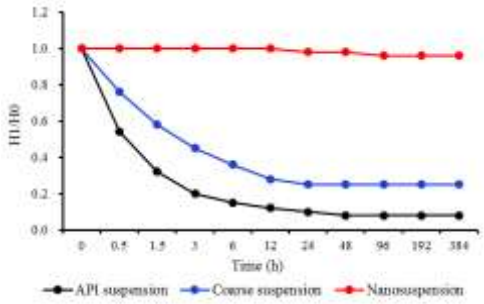

Figure 2. Sedimentation curve of drug suspension. (API referred to active pharmaceutical ingredient).

Effect of Chinese herbal compound nano-suspension on PASI score of psoriatic mouse skin lesion

As the mice in the NC group had no skin lesions, PASI scores of erythema, scale, and infiltration thickening of skin lesions in other groups were scored. In Figure 3, except for the NC group, PASI scores of erythema, scale, and infiltration thickening of skin lesions in other groups showed an increasing trend with time, among which the fastest was the PsM group. According to the comparison, on day 1, there was no significant difference in PASI scores of PsM, TrG, YX1, and Nano-YX1 groups ($P>0.05$). The scale, infiltration thickening degree, and the total

PASI score on days 3, 5, and 7 in the PsM group were greatly higher than those in the TrG, YX1, and Nano-YX1 groups (P<0.05), and the PASI score of erythema in PsM group was remarkably higher than that of YX1 and Nano-YX1 groups (P<0.05). The sequence of PASI scores from high to low was the TrG group, YX1 group, and Nano-YX1 group. There were considerable differences in PASI scores among all the groups (P<0.05).

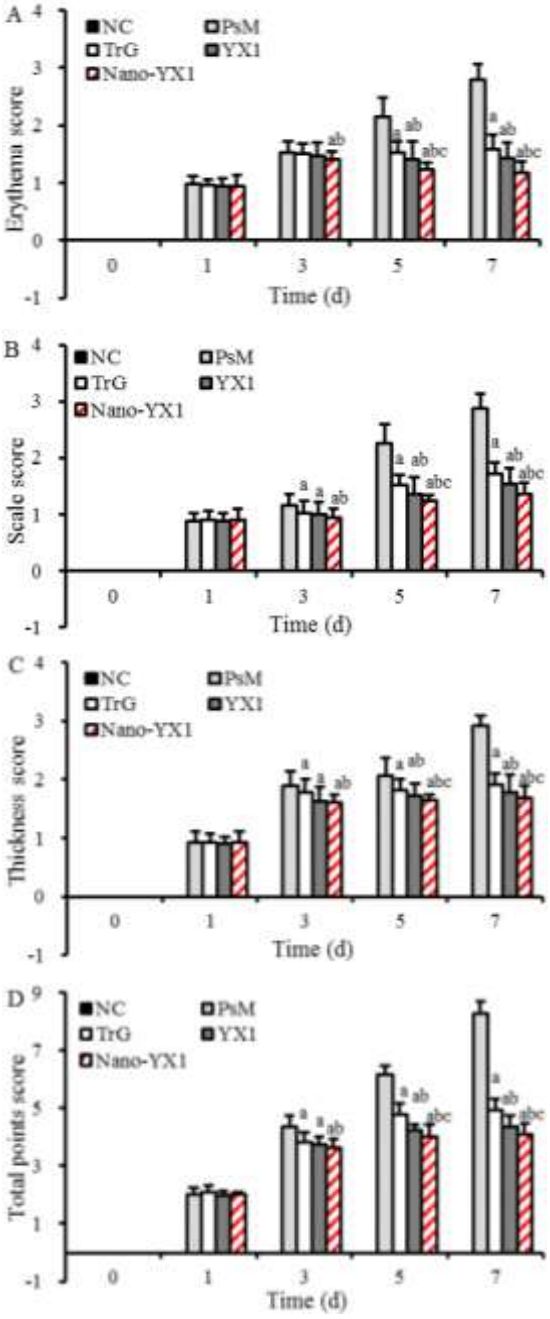

Figure 3. PASI scores of skin lesions in each group. (A: erythema score; B: scale score; C: infiltration thickening score; D: total score; compared with PsM group, [a]P<0.05; compared with TrG group, [b]P<0.05; compared with YX1 group, [c]P<0.05).

Effect of Chinese herbal compound nano-suspension on the pathological changes of psoriatic mice skin lesions

The pathological changes in skin or skin lesions were compared in each group. In Figure 4A, the surface skin of mice in the NC group was very thin without stratification, and there were no obvious infiltrating lymphocytes subcutaneously. The epidermis of mice in the PsM group showed evident stratification and thickening, and many infiltrating lymphocytes appeared subcutaneously. The epidermis of mice in the TrG, YX1 and Nano-YX1 groups was thickened, but the thickness was signally less than that in the PsM group. The stratification was manifestly improved, and the subcutaneous infiltrating lymphocytes were relatively fewer. In Figure 4B, compared with the NC group, the vertical epidermal thickness of mice in PsM, TrG, YX1, and Nano-YX1 groups was observably increased (P<0.05). Compared with the PsM group, the vertical epidermal thickness of mice in the TrG, YX1, and Nano-YX1 groups was markedly reduced (P<0.05). In these groups, the decrease of vertical epidermal thickness in the Nano-YX1 group was the most obvious, and its significance was smaller than that in TrG and YX1 groups (P<0.05).

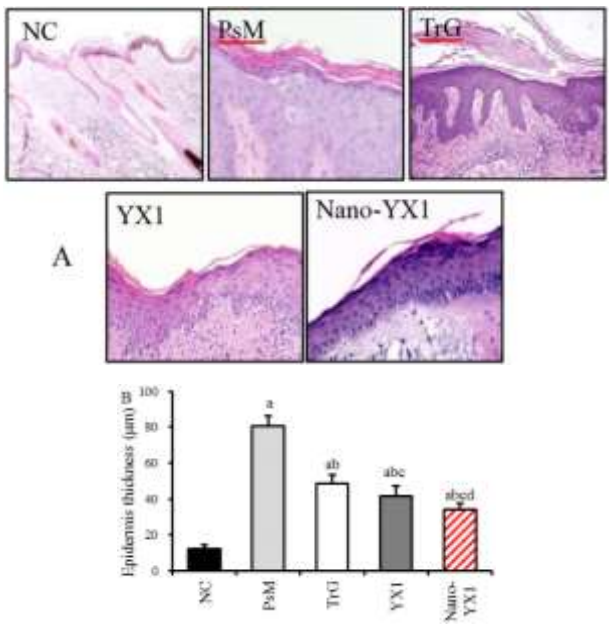

Figure 4. Pathological changes of skin lesions in each group. (A: HE staining of skin lesions with a magnification of ×200; B: measurement results of epidermis vertical thickness; compared with NC group, [a]P<0.05; compared with PsM group, [b]P<0.05; compared with TrG group, [c]P<0.05; compared with YX1 group, [d]P<0.05).

Effects of Chinese herbal compound nano-suspension on messenger ribonucleic acid (mRNA) expression of STAT3 and STAT5 in psoriatic mice skin lesions

The mRNA expression levels of STAT3 and STAT5 in skin lesions were compared among all the groups. In Figure 5, compared with the NC group, the mRNA expression levels of STAT3 and STAT5 in skin lesions of mice in PsM, TrG, YX1, and Nano-YX1 groups were notably increased (P<0.05). Compared with the PsM group, the mRNA expression levels of STAT3 and STAT5 in TrG, YX1, and Nano-YX1 groups were remarkably decreased (P<0.05). Compared with the TrG group, the mRNA expression levels of STAT3 and STAT5 in skin lesions of mice in the YX1 group and Nano-YX1 group were observably decreased (P<0.05). Compared with the YX1 group, the mRNA expression levels of STAT3 and STAT5 in skin lesions of mice in the Nano-YX1 group were greatly decreased (P<0.05).

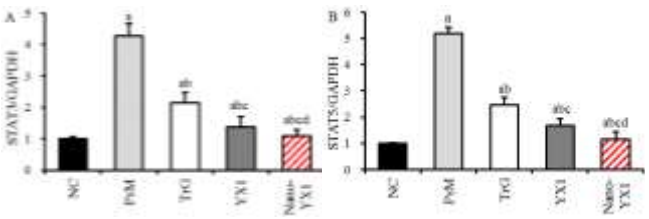

Figure 5. Comparison of STAT3 and STAT5 mRNA expression levels in skin lesions of each group. (A: mRNA expression of STAT3; B: mRNA expression of STAT5; compared with NC group, [a]P<0.05; compared with PsM group, [b]P<0.05; compared with TrG group, [c]P<0.05; compared with YX1 group, [d]P<0.05).

Effects of Chinese herbal compound nano-suspension on STAT3 and STAT5 protein expression in psoriatic mice skin lesions

The protein expression levels of STAT3, STAT5, p-STAT3, and p-STAT5 in skin lesions were compared among all the groups. In Figure 6, there was an insignificant difference in STAT3 and STAT5 protein expression levels in skin lesions (P>0.05). Compared with the NC group, the protein expression levels of p-STAT3 and p-STAT5 in skin lesions of PsM, TrG, YX1, and Nano-YX1 groups were increased (P<0.05). Compared with the PsM group, the protein expression levels of p-STAT3 and p-STAT5 in the TrG group, YX1 group, and Nano-YX1 group were decreased (P<0.05). Compared with the TrG group, the protein expression levels of p-STAT3 and p-STAT5 in skin lesions of mice in the YX1 group and Nano-YX1 group were significantly decreased (P<0.05). The p-STAT3 and P-STAT5 protein expression levels in skin lesions of mice in the Nano-YX1 group were significantly decreased compared with the YX1 group (P<0.05).

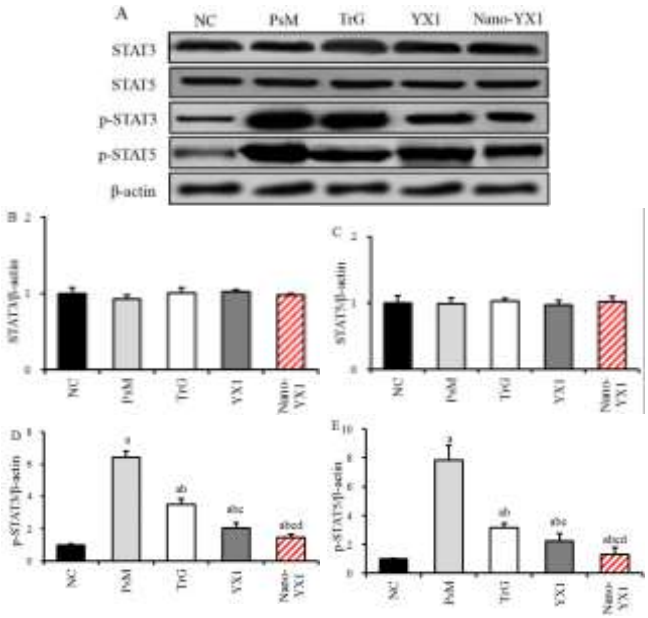

Figure 6. Comparison of STAT3, STAT5, and phosphorylated protein expression levels in skin lesions of mice in each group. (A: the Western blot image; B: the protein expression of STAT3; C: the protein expression of STAT5; D: the protein expression of p-STAT3; E: the protein expression of p-STAT5; compared with NC group, [a]P<0.05; compared with PsM group, [b]P<0.05; compared with TrG group, [c]P<0.05; compared with YX1 group, [d]P<0.05).

Psoriasis is a chronic inflammatory skin disease with genetic background, which is closely related to specific immune response abnormalities (17). The current pathogenesis of psoriasis is still unclear, but it has such characteristics as stubborn refractory and a high recurrence rate (18). Hence, to improve the clinical cure rate of psoriasis, both Chinese and western medicine have investigated widely on the disease. In the category of traditional Chinese medicine, psoriasis belongs to the “BaiBi” of Chinese traditional medicine, which has the characteristics of blood heat accumulation, IL-8 of blood stasis, and wind-evil penetration (19). According to the above characteristics, the Chinese herbal compound of clearing heat, cooling blood, and detoxifying is prepared, which can manifestly improve the clinical

cure rate of psoriasis. Currently, to improve the time of exposure and increase the efficacy of drugs, the proposed nano-drug delivery system helps to solve the crucial problems of a short half-life period and poor bioavailability (20).

The HPH method was used to prepare the Chinese herbal compound Yinxia-1 nano-suspension for the treatment of psoriasis, and the therapeutic effects were compared with the API of YinXie No.1 (21). The results showed that the average particle size of YinXie No. 1 was only 1.7±0.6μm, and the distribution was uniform, which fully met the requirements. According to the sedimentation curve, the volume of nano-suspension didn't change evidently with the increase of time compared with the API. Consequently, the nano-suspension prepared by the HPH method had a good suspension state, and it was very stable. This result was consistent with that of Makjaruskul and Sripanidkulchai (22). To evaluate the therapeutic effect of the Chinese herbal compound nano-suspension, a psoriasis mouse model was prepared and treated by gavage. The HE sections of psoriasis mice were prepared to observe the pathological changes. After the PASI score was completed, the lesions of psoriasis mice were increased notably, and so was the PASI score. Both the API decoction of YinXie No.1 and the nano-suspension helped improve the symptoms of skin lesions in mice, and the improvement of nano-suspension was more obvious. The prepared YinXie No. 1 nano-suspension could improve the therapeutic effect of drugs.

The global incidence of psoriasis is about 3%, and the genetic effect of multiple genes is an important pathogenic factor (23). Psoriatic lesions are infiltrated by the abnormal T lymphocytes, and the abnormally activated T lymphocytes secrete a variety of cytokines, which can participate in the process of psoriasis through the JAKs/STATs pathway (24,25). The JAKs/STATs pathway is a vital cytokine signal transduction pathway in the body, through which most cytokines can play their biological roles (26). At present, the JAKs/STATs pathway is proven to be involved in the pathological process of psoriatic vascular dysplasia (27). JAK is a cytoplasmic soluble tyrosine-protein kinase (28). STAT has 7 family members, namely STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. Both STAT3 and STAT5 are substrates of JAK2 (29-31). The expressions of STAT3 and STAT5 in the JAKs/STATs pathway in psoriatic mice were further detected. The results showed that the mRNA expression levels of STAT3 and STAT5 in psoriatic mice were abnormally increased. However, the mRNA expression levels of STAT3 and STAT5 were markedly decreased after treatment with the API decoction of YinXie No.1 and the nano-suspension, and the effect of nano-suspension was obvious. Moreover, the protein expression levels of p-STAT3 and p-STAT5 in psoriatic mice were also abnormally increased. The API decoction of YinXie No.1 and the nano-suspension could decrease the protein expressions of p-STAT3 and p-STAT5.

Conclusions

The Chinese herbal compound YinXie No. 1 nano-suspension was successfully prepared by HPH method, and the nano-suspension had better stability than API. After psoriasis mice have treated with API decoction of Chinese herbal compound Yinxie No. 1 and nano-suspension, both drugs were helpful to signally improve the pathological changes of psoriasis mice, and the improvement effect of YinXie No. 1 nano-suspension was obvious. Chinese herbal compound YinXie No.1 helped inhibit the expression and phosphorylation of STAT3 and STAT5 in the JAKs/STATs pathway. The signal transduction of some cytokines was interfered with, which played a role in the treatment of psoriasis. Nevertheless, only the problem that whether the JAKs/STATs pathway is involved in the treatment of psoriasis by Chinese herbal compound is explored in the experiment, and the mechanism of the JAKs/STATs pathway and the proliferation, differentiation, and inflammation of keratinocytes in psoriasis isn't investigated. Meanwhile, there is no in-depth exploration of specific cytokines. Therefore, it is expected to supply the above contents at the cellular level in the future. The results of this experiment can provide a basis for the selection of clinical treatment for psoriasis.

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

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