Effectiveness of Xiaoyin Jiedu granules in the treatment of psoriasis vulgaris in patients with blood-heat symptom patterns in terms of Traditional Chinese Medicine

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OBJECTIVE: To evaluate the effectiveness of Xiaoyin Jiedu granules (XYJD) in the treatment of psoriasis vulgaris (PSV) in patients with blood-heat symptom patterns in terms of Traditional Chinese Medicine (TCM). We also aimed to identify the possible underlying immunological mechanism.

METHODS: Twenty-five PSV patients with BHP and ten normal controls were enrolled from January 1, 2015 to December 31, 2016. Patients were randomly assigned to either the XYJD group (15 cases) or the placebo group (10 cases), in which patients were treated with XYJD granules or a placebo, respectively. Additionally, albolene was used to relieve skin dryness in these two groups. The psoriasis area and severity indexes, dermatology life quality indexes and itching scores were assessed at the end of the 2nd, 4th and 8th week of treatment. The number of peripheral blood T helper (Th) 9, Th17 and regulatory T cells (Tregs) and the mRNA and protein expression levels of PU.1, RAR-related orphan receptor (ROR)-yt, forhead box protein 3 (Foxp3), interleukin (IL)-9, IL-17, IL-23 and IL-10 in the control and experimental groups were compared before and after treatment.

RESULTS: Psoriasis area and severity indexes, dermatology life quality indexes and itching scores of patients in the XYJD group were significantly lower than those in the placebo group, whereas dermatology life quality indexes were significantly higher. In comparison with the placebo group, XYJD granules significantly reduced the number of Th17 cells and the mRNA and protein expression levels of Th17-related ROR-yt, IL-17, IL-22 and IL-23 in the peripheral blood and reduced the number of Th9 cells and the mRNA and protein expression levels of Th9-related PU.1 and IL-9.

CONCLUSION: XYJD granules were effective against PSV in patients with BHP by reducing the number of Th9 and Th17 cells and the levels of their related cytokines.
INTRODUCTION

Psoriasis is a common and chronic inflammatory skin disease characterized by the presence of erythema and scales. Genome-wide scans for psoriasis-associated genes have identified predominantly immune-related genes, which provides a mechanistic link between genetics and immunity. The main pathogenesis is that inflammatory cytokines secreted by the innate immune system activate T lymphocytes, which infiltrate into the epidermis and form a negative complex feedback loop with keratinocytes and vascular endothelial cells, which results in psoriatic pathophysiological abnormalities. One of the predominant types of proinflammatory cells are T helper (Th) 17 cells, which produce interleukin (IL)-17 and play a key role in psoriasis inflammation. The differentiation process of Th17 cells is controlled by the RAR-related orphan receptor (ROR)-γt. IL-23 maintains the expansion and survival of Th17 cells and the IL-23/Th17 axis plays an important role in the development and progression of psoriasis. Th9 cells, another subtype of CD4+ T cells, were first identified in 2008 by polymerase chain reaction (PCR) and intracellular staining. The transcription factor PU.1 regulates the differentiation program of Th9 cells. Th9 cells predominantly produce IL-9 and small amounts of IL-10. IL-9 can deteriorate the skin lesion in psoriasis and accelerate the inflammation-related skin damage. Some researchers believe that the aberrant activation of Th9 cells may contribute to the development and progression of psoriasis.

Similar to Th17 cells, regulatory T cells (Tregs) differentiate from naive CD4+ T cells. These two subsets often serve opposite roles during the differentiation and inflammatory processes, whereas they remain balanced under normal conditions. The balance of Th17/Tregs is essential to maintain immune homeostasis, and an imbalance is one of the mechanisms that contribute to the development of immune disorders. Forkhead box protein 3 (Foxp3) is critically involved in the differentiation and function of Tregs. IL-10, the predominant product of Tregs, can inhibit the proliferation of inflammatory T cells and reduce the damage caused by immune overreaction.

Xiaoyin Jiedu (XYJD) decoction was developed by Professor Jin Qifeng, a national-level traditional Chinese senior physician at the Dongzhimen Hospital who is affiliated with the Beijing University of Chinese Medicine. The aim of this study was to evaluate the effectiveness of XYJD granules in the treatment of psoriasis vulgaris (PSV) in patients with blood-heat pattern (BHP) in terms of Traditional Chinese Medicine (TCM). We also investigated the possible underlying immunological mechanism.

MATERIALS AND METHODS

Ethical approval
The study was approved by the Beijing University of Chinese Medicine Ethics Committee (Approval No. BJZYDX-LL-2014017) and conducted in accordance with the principles of the Declaration of Helsinki. Informed consent forms were voluntarily signed by all participants before their enrollment.

Participant information
Thirty patients with PSV were recruited from the Dermatology Department of Dongzhimen Hospital, which is affiliated with Beijing University of Chinese Medicine, during the period from January 1, 2015 to December 31, 2016. Ten control patients were defined as not having any skin diseases.

Diagnosis criteria and symptom pattern identification standard
Diagnostic criteria of PSV strictly followed standards listed in China Clinical Dermatology. TCM symptom pattern identification criteria met the demands of the standard for psoriasis (BHP) identification set by the Skin Disease Branch Committee of the China Association of Chinese Medicine in 2013. The primary symptoms included (a) bright red skin lesions and (b) continuous emerging and expanding of new lesions. Minor symptoms included (a) vexation and short temper, (b) deep-colored urine, (c) deep red or purple tongue and (d) taut-slippery or rapid pulse. Patients with all primary symptoms and one minor symptom were confirmed to have the BHP.

Inclusion criteria
Patients who met the Western medicine and TCM diagnostic criteria were enrolled. They were aged between 18 and 65, and they had not taken drugs such as tretinoin, immunosuppressors, tripterygium glycosides or glucocorticoids, received narrow-band ultraviolet B (NB-UVB) therapy in the previous 3 months or taken any medications for external use in the previous 2 weeks.

Exclusion criteria
The following patients were excluded from this study: pregnant or breastfeeding women, those with serious primary cardiovascular, cerebrovascular, liver or kidney diseases and those with psychiatric conditions.

Treatments
Thirty portions of herbal granules including 15 placebos were numbered from 1 to 30 according to strati-
fied block randomization. The 30 patients were randomly divided into the two following groups. Patients in the XYJD group took the XYJD granules twice a day [ingredients: Shuiniujiao (Cornus Bucha) 20 g, Dihuang (Radix Rehmanniae) 15 g, Mudanpi (Cortex Moutan Radicis) 10 g, Chishao (Radix Paeoniae Rubra) 10 g, Rendongteng (Honeysuckle Stem) 20 g, Baixianpi (Cortex Dictamni Radicis) 10 g, Tufuling (Rhizoma Smilacis Chinae) 15 g and Gancao (Radix Glycyrrhizae) 6 g]. Those in the placebo group took a placebo (ingredient: amyloextrin) in a similar granule form, twice a day. Both groups received treatment for 8 weeks. The above drugs were provided by Kang Rentang Pharmaceutical Co., Ltd. (Beijing, China). Alboline was externally applied in patients with itchy dry skin.

The peripheral blood of each patient was collected at the beginning and end of the experiment for flow cytometry analysis. Isolated peripheral blood mononuclear cells (PBMCs) were frozen at −80 °C.

**Clinical index evaluation**

Psoriasis area and severity indexes (PASI), dermatology life quality indexes (DLQI), and itching scores were assessed on the 2nd, 4th and 8th week of treatment. Blood routing and hepatic and renal function examinations were performed at the beginning and end of the experiment to avoid untoward effects. All outcome measures were assessed by an independent researcher who was blinded to the treatments.

**Flow cytometry**

Flow cytometry was performed to compare the differentiation levels of Th9, Th17 and Treg cells in the peripheral blood of the two groups before and after the experiment. For the detection of Th9 and Th17 cell differentiation, fresh peripheral blood was incubated with a PE-labeled anti-human CD4 antibody (BD Biosciences, San Jose, CA, USA) for 30 min and then stimulated with phorbol myristate acetate (50 ng/mL), ionomycin (1 μg/mL) and monensin (2 μM) for 4 h at 37 °C. The cells collected as described above were fixed and permeabilized. The cells were washed twice and incubated with FITC-labeled anti-human IL-9 (BD Biosciences, San Jose, CA, USA) and FITC-labeled anti-human IL-17A (BD Biosciences, San Jose, CA, USA) antibodies in the dark for 2 h. For the detection of Treg differentiation, PBMCs were stained with a FITC-labeled anti-human CD4+CD25 antibody (BD Biosciences, San Jose, CA, USA) for 30 min. After fixation and permeabilization, cells were incubated with a PE-labeled anti-human Foxp3 antibody (BD Biosciences, San Jose, CA, USA). All stained cells were resuspended in 200 μL washing buffer and analyzed using a flow cytometer (EPICS XL/FC500/Alter, Beckman Coulter).

**RNA purification and quantitative PCR**

Total RNA was extracted from PBMCs using the RNA-prep pure Tissue Kit (Qiagen, Chatsworth, CA, USA) and reversed transcribed using the PrimeScript™ RT Reagent Kit with a gDNA Eraser (TaKaRa, Shiga, Japan). Quantitative PCR was performed using SYBR® Premix Ex Taq™ II (TaKaRa, Shiga, Japan) following the manufacturer’s protocols. The PCR reaction conditions were as follows: pre-degeneration at 95 °C for 10 min, denaturation at 95 °C for 5 s, and annealing at 60 °C for 40 s for a total of 40 cycles. Gene expression was normalized to the housekeeping gene glyceraldehyde-phosphate dehydrogenase (GAPDH). The primers used were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and their sequences were as follows: PU.1, forward (F) 5'-CCGAGATGGAT-3' and reverse (R) 5'-GCATCTGCTCAGCTCCAT-3'; ROR-γt, F 5'-CTGGAAGCTCATCGGCAAAAG-3' and R 5'-TTTTTCACTGCTGCTACACA-3'; Foxp3, F 5'-TCTTCTTGGACC-CCATGCC-3' and R 5'-AAATGTGGGCGTCTGTG-3'; IL-9, F 5'-CCAGCTTCCAAGCTCAGCT-GC-3' and R 5'-TGCTTGTGGATGTGGATCATCT-3'; IL-10, F 5'-CCCGAGATGGCCTCAGAG-TGT-3' and R 5'-GGCTTTAGTCTGTGCTT-GTT-3'; IL-17, F 5'-GGGCTTGAGGCCATA-GTGA-3' and R 5'-CAGGTTGAGTGTCATG-3'; IL-22, F 5'-GATAAATAAACAACAGAT-GTCAGGCTC-3' and R 5'-GATCGCTTTAA-TCTCTCCACTCCTC-3'; and IL-23, F 5'-TGTCCTCCTGATAGCCCTGTGG-3' and 5'-TGAGGCTCGAGAGGGATT-3'.
USA). After washing three times with tris-buffered saline containing tween-20 (TBST), the PVDF membranes were incubated with goat anti-rabbit IgG (H+L)-HRP (Jackson, West Grove, PA, USA) or goat anti-mouse IgG (H+L)-HRP (Jackson, West Grove, PA, USA) secondary antibodies at room temperature for 1 h. Following additional washes, the protein bands were detected by the chemical luminescence method (ECL, Santa Cruz, CA, USA) and imaged using a gel image system ver.4.00. GAPDH was used as a loading control.

Statistical analysis
Data are shown as the mean ± standard deviation (± s). Data were analyzed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). The Kruskal-Wallis test was conducted to compare multiple groups, while Dunn’s multiple comparison test was used for comparison of pairs. A two-tailed Mann-Whitney U test was used for independent data, while a two-tailed Wilcoxon matched-pairs signed-rank test was used to test paired data. P < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics
Twenty-five patients completed this study; the male to female ratio was 4:1. Five patients in the placebo group were excluded from the study because of aggravation and the total drop-out rate was 16.7%. The patient characteristics are shown in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (years, s ± μ)</th>
<th>Recurrence (case in total)</th>
<th>Course (years, s ± μ)</th>
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</thead>
<tbody>
<tr>
<td>XYJD</td>
<td>15</td>
<td>46 ± 13</td>
<td>14/15</td>
<td>17 ± 14</td>
</tr>
<tr>
<td>Placebo</td>
<td>10</td>
<td>51 ± 13</td>
<td>10/10</td>
<td>22 ± 12</td>
</tr>
</tbody>
</table>

Notes: Twenty-five patients with blood-heat pattern of psoriasis vulgaris were involved in this study. Fifteen patients in XYJD group took the Xiaoqin Jiedu granules twice a day, 10 patients in placebo group took a placebo (ingredient: amylodextrin) in a similar granule twice a day. Both groups received treatment for 8 weeks. XYJD: Xiaoqin Jiedu group. Placebo: placebo group. There was no difference in age (P = 0.38 > 0.05), recurrence ratio (P = 0.91 > 0.05) and the course of disease (P = 0.23 > 0.05) between XYJD group and placebo group.

XYJD granules decreased PASIs and itching scores and improved the quality of life
Before treatment, there was no significant difference between the two groups in PASIs (P = 0.62 > 0.05), DLQIs (P = 0.62 > 0.05) and itching scores (P = 0.21 > 0.05). After treatment, PASIs, itching scores and DLQIs in the XYJD group gradually declined (Figure 1A, B, C). Conversely, there were no significant changes in the placebo group (Figure 1A, C) and itching scores gradually increased (Figure 1B).

XYJD granules decreased the differentiation ratio of Th9 and Th17 cells
Flow cytometry results showed that the number of Th9 and Th17 cells was lower after treatment with XYJD granules than before, but there was no change in the placebo group (Figure 2A, B). Figure 2C shows that there was no significant difference between the number of Tregs in XYJD group and in placebo group before and after treatment.

XYJD granules decreased mRNA and protein expression levels of Th9-related PU.1 and IL-9 and Th17-related RORγt, IL-17, IL-22 and IL-23 in peripheral blood
Quantitative PCR and western blot analysis revealed that the mRNA and protein levels of PU.1 and RORγt, specific transcription factors of Th9 and Th17 cells, respectively, and IL-9, IL-17, IL-22 and IL-23 were significantly decreased in the XYJD group and remained unchanged in the placebo group. Conversely, the mRNA and protein levels of Foxp3, the specific transcription factor of Tregs and IL-10 decreased in both groups. (Figures 3, 5)

The difference (d) value levels of PU.1, RORγt, IL-9, IL-17, IL-22 and IL-23 mRNAs before and after treatment in the XYJD group were higher than in the placebo group (Figure 4). Conversely, no significant difference was observed in the d value of Foxp3 and IL-10 mRNA expression between the two groups.

DISCUSSION
Blood heat is the main cause of psoriasis in TCM theory. Studies suggest that T lymphocytes and their cytokines might be the material basis of “heat-toxin” in psoriasis.
Figure 2 Comparison of Th9, Th17, and Treg Subsets in each group
The number of Th9, Th17, and Tregs in XYJD before and placebo before were higher ($P < 0.01$ or $P < 0.05$) than the normal controls. A: after 8 weeks of treatment. There was no statistical difference in placebo before ($P > 0.05$) and after. The number of Th9 cells decreased in XYJD after ($P < 0.05$) compared with XYJD before. B: there was no statistical difference in placebo before ($P > 0.05$) and after treatment; the number of Th17 cells significantly decreased in XYJD after ($P < 0.001$) compared with XYJD before. C: there was no statistical difference in placebo before ($P > 0.05$) and after; there was no significant difference in the number of Tregs in XYJD before and placebo before ($P > 0.05$) compared with XYJD after and placebo after. XYJD: Xiaoyin Jiedu group, Placebo: placebo group, PBMCs: peripheral blood mononuclear cells, Th: Th helper, Tregs: regulatory T cells.

Figure 3 Comparison of the mRNA expression levels of PU.1, ROR-γt, Foxp3, IL-9, IL-17, IL-22, IL-23, and IL-10 in peripheral blood in each group
The mRNA expression levels of PU.1, ROR-γt, Foxp3, IL-9, IL-17, IL-22, and IL-23 in XYJD before and placebo before were higher ($P < 0.01$ or $P < 0.05$ or $P < 0.001$) compared with normal controls. A, B, C, D, E, F, G: after 8 weeks of treatment; the mRNA expression levels of PU.1, ROR-γt, IL-9, IL-17, IL-22, and IL-23 significantly decreased in XYJD after ($P < 0.001$ or $P < 0.01$) compared with XYJD before, and there was no statistical difference in placebo before ($P > 0.05$) compared with placebo after; C, H: the mRNA expression levels of Foxp3 and IL-10 decreased in XYJD after and placebo after ($P < 0.01$ or $P < 0.05$) compared with XYJD before and placebo before. XYJD: Xiaoyin Jiedu group, Placebo: placebo group, mRNA: messenger Ribonucleic Acid; IL: interleukin, ROR-γt: RAR-related orphan receptor (ROR)-γt; Foxp3: forkhead box protein 3.

...as the material basis of ‘internal blood heat and toxic factors’.
Modern pharmacological studies show that Shuiniujiao (Cornu Bubali), similar to rhinoceros horn, has anti-inflammatory effects and reduces the permeability of capillaries. Additionally, Dihuang (Radix Rehmanniae), Tufuling (Rhizoma Smilacis Chinas) and Gancao (Radix
**Figure 4** Comparison of the d value levels of PU.1, ROR-γt, Foxp3, IL-9, IL-17, IL-22, IL-23, and IL-10 mRNAs in XYJD and placebo groups.

A, B, D, E, F, G: within 8 weeks of treatment, the d value levels of PU.1, ROR-γt, IL-9, IL-17, IL-22, and IL-23 mRNAs in placebo group were lower \((P < 0.01 \text{ or } P < 0.05)\) compared with the XYJD group; C, H: the d value levels of Foxp3 and IL-10 in the placebo group were the same \((P > 0.05)\) compared with the XYJD group. XYJD: Xiaoyin Jiedu group; Placebo: placebo group; d value: difference value; mRNA: messenger Ribonucleic Acid; IL: interleukin; ROR-γt: RAR-related orphan receptor (ROR)-γt; Foxp3: forkhead box protein 3.

**Figure 5** PU.1, ROR-γt, Foxp3, IL-9, IL-17, IL-22, IL-23, IL-10 and GAPDH protein expression in groups.


**Glycyrrhiza* inhibit epithelial mitosis, promote keratinocyte differentiation and inhibit proliferation, whereas *Rendongteng* (*Honeysuckle Stem*), *Chishao* (*Radix Paeoniae Rubra*) have anti-inflammatory and anti-viral effects. Furthermore, *Mudanpi* (*Cortex Moutan Radicis*) and *Chishao* (*Radix Paeoniae Rubra*) suppress keratinocyte proliferation and secrete IL-8, which is stimulated by TNF-α.

PSV is a dendritic cell and T-cell-mediated disease with complex feedback loops from antigen-presenting cells, neutrophilic granulocytes, keratinocytes, vascular endothelial cells and the cutaneous nervous system. As one of the main initiating cells, dendritic cells secrete IL-23 and other cytokines, which can induce the activation and differentiation of Th17 cells. Once activated, Th17 cells produce several specific cytokines, such as IL-17 and IL-22. The former accelerates inflammation in psoriatic lesions and the latter inhibits the expression of keratinocyte differentiation genes and promotes keratinocyte proliferation. These changes lead to hyperkeratosis and parakeratosis, which are pathological manifestations of psoriasis. Tregs oppose Th17 cell function and differentiation. Under normal circumstances, there is a balance in Treg/Th17 cells, and the disruption of this balance occurs in autoimmune diseases. IL-10, TGF-β and IL-35 are the predominant products of Tregs. It was found that Foxp3+ Treg cells can be easily transformed into cells secreting IL-17A in psoriatic lesions. IL-9- produced by Th9 cells participates in the expression of is associated with Th17-related inflammation in psoriasis. Some scholars believe that it works through its own autocrine and paracrine activities.

In this study, we found that XYJD granules significantly reduced PASIs, DLQIs and itching scores in psoriasis patients with the BHP. Our experimental results show that XYJD granules can not only significantly reduce the number of Th17 cells and the mRNA and protein expression levels of Th17-related ROR-γt, IL-17, IL-22 and IL-23 in the peripheral blood, but they can also reduce the number of Th9 cells and mRNA and protein expression levels of Th9-related PU.1 and IL-9.

In summary, XYJD granules effectively remove heat from blood and toxic materials. This involves reducing
the number of Th9 and Th17 cells and suppressing the mRNA expression of various cytokines and inflammatory mediators associated with Th9 and Th17.

REFERENCES