Xiaoyin Jiedu Granules may alleviate psoriasis-like skin diseases in mice by regulating sphingosine 1-phosphate receptor expression and reducing Th17 cells

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A R T I C L E   I N F O

A B S T R A C T

Sphingosine-1-phosphate (S1P) is associated with the onset and severity of psoriasis, a chronic inflammatory skin disease linked to innate and adaptive immune responses. This study explores the therapeutic effect of Xiaoyin Jiedu Granules, a combination of traditional Chinese medicines, on psoriasis-like skin lesions in mice and the underlying mechanism. We used imiquimod (IMQ) to induce psoriasis-like dermatitis in mice; the effects of Xiaoyin Jiedu Granules on S1P receptors (S1PRs) were investigated using histology and immunohistochemistry. The effects of Xiaoyin Jiedu Granules on the proliferation, differentiation, and activation of the NF-κB pathway in keratinocytes were verified using quantitative polymerase chain reaction (qPCR) and western blotting analyses. CD4$^+$Th17 cells were screened using flow cytometry; the effects of Xiaoyin Jiedu Granules on the differentiation of Th17 cells and the content of related inflammatory factors were also verified. S1PR1-5 was highly expressed in psoriatic lesions. Xiaoyin Jiedu Granules significantly inhibited the secretion of proliferation-related proteins (K6, K16, K17, and IL-36γ) and proinflammatory cytokines (IL-17 and IL-22), transformation of Th17 cells, and activation of the NF-κB pathway and effectively alleviated IMQ-induced psoriasis-like dermatitis. Overall, our findings indicate that Xiaoyin Jiedu Granules have anti-inflammatory activity against S1PR expression, keratinocytes, and immune cells and can therefore mitigate psoriasis. Inhibiting the expression of S1PRs may be an effective treatment strategy against psoriasis.

1. Introduction

Psoriasis (PSO) is an immune-mediated chronic relapsing inflammatory disease that affects the skin and/or joints, with a global incidence of approximately 2% [1], affecting approximately 0.47% of the Chinese population [2]. PSO causes a significant burden on patients and caregivers because it significantly reduces their quality of life [3]. Data on the pathogenesis of PSO remain limited; however, previous findings suggest that the mutual inflammatory enhancement between T helper cells (Th) 17 and keratinocytes (KCs) influence the development of PSO [1].

In recent years, lipid metabolism dysfunction in patients with PSO has been reported [4–6]. Studies have shown a significant increase in the concentration of sphingosine-1-phosphate (S1P), a type of sphingolipid, in the serum of patients with PSO [7].

Ceramide, another sphingolipid, is hydrolyzed into sphingosine, which is, in turn, phosphorylated to produce S1P, which binds to five specific G protein-coupled receptors (S1PR1-5), via which it exerts various physiological effects. Ceramides play an essential role in maintaining the skin's barrier function, the loss of which is associated with angiogenesis and a persistent inflammatory state. The S1P/S1PR axis has garnered attention in autoimmune disease research and treatment. S1PR gene deletion can reduce the inflammatory response and epidermal proliferation in skin lesions induced by imiquimod (IMQ) in mice and in transplanted psoriasis lesions [8]. Therefore, the S1P signaling pathway is a potential treatment strategy against PSO [9].

Traditional Chinese medicine (TCM) has a high penetration rate among patients with PSO. TCM considers that "blood heat" (i.e., bleeding and burning sensations) is the primary cause of PSO, and that this condition leads to the bright red skin lesions and blood vessel proliferation observed in PSO. Xiaoyin Jiedu Granules, which have a significant therapeutic effect on PSO, are a combination of TCMs developed by Dr. Jin Qifeng at the Dongzhimen Hospital of Beijing University of Traditional Chinese Medicine. Xiaoyin Jiedu Granules, along with the two disassembled decoctions, Liangxue ("cooling blood") and Jiedu ("detoxification") formulas, can reduce the number of Th17 cells in the peripheral blood of patients with PSO, reduce the secretion of inflammatory cytokines such as IL-23 and IL-17, and inhibit KC proliferation [10]. However, whether these decoctions alleviate PSO by interfering with S1PR expression and Th17 cell differentiation remains to be determined. Toward this end, this study explores whether these decoctions have a regulatory effect on the inflammatory signaling pathways associated with S1PRs and Th17 through *in vitro* experiments to determine the effect and underlying mechanism of Xiaoyin Jiedu Granules and the disassembled formula on PSO-like skin lesions.

2. Materials and methods

2.1. Mouse model

Thirty clean-grade healthy female inbred BALB/c mice (8 weeks old, weighing 18 ± 2 g) were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd.: animal certificate number: SCXK (Beijing) 2016-0006. The mice were kept in a specific pathogen-free-grade animal room in the Key Laboratory of Dongzhimen Hospital, Beijing University of Traditional Chinese Medicine, with free access to food and water and adaptive feeding for 1 week.

2.2. Drugs

The composition of Xiaoyin Jiedu Granules is as follows: 30 g of Shuiniujiao, 15 g of Shengdihuang, 15 g of Danpi, 15 g of Chishao, 15 g of Baihuasheshecao, 15 g of Quanshen, 20 g of Zicao, 10 g of Shenghuaihua, 15 g of Tufuling, 15 g of Daqingye, and 20 g of Rendongteng. The Liangxue decoction was as follows: 30 g of Shuiniujiao, 15 g of Shengdihuang, 15 g of Danpi, 15 g of Chishao, 20 g of Zicao, 10 g of Shenghuaihua. The Jiedu decoction was as follows: 15 g of Baihuasheshecao, 15 g of Quanshen, 15 g of Tufuling, 15 g of Daqingye, and 20 g of Rendongteng. The drugs were soluble granules produced by Beijing Kangrentang Pharmaceutical Co., Ltd. Distilled water at 80 °C was added to the granules to fully dissolve them for later use.

The 5% IMQ cream was from 3 M Health Care Ltd. (C14200158360), whereas the Vaseline was obtained from Tianjin Shuangsheng Chemical Factory.

2.3. Treatment and sampling

The back fur of the mice was shaved off, and the mice were randomly divided into five groups (six mice per group): Control, Model, Total prescription, Liangxue decoction, and Jiedu decoction. Except for the control group (treated with the same amount of Vaseline), all groups were smeared with 50 mg of an ointment containing 5% IMQ once a day for five consecutive days and immediately treated with medicine (gavage), with either the whole prescription of Xiaoyin Jiedu granules, Liangxue decoction, or Jiedu decoction being administered to the corresponding group. The treatment dosage was 10 ml/kg, twice daily for five days. The control and PSO model groups were intragastrically administered the same amount of normal saline. After 4 h of IMQ induction on day 5 of treatment, blood was collected from the abdominal aorta, the serum was separated, and the mice were sacrificed via neck dislocation. The back skin and spleen were also collected for histological and immunostaining analysis. This study was approved by the Animal Ethics Committee of Dongzhimen Hospital, Beijing University of Traditional Chinese Medicine, and complied with the ethical requirements for animal experiments (approval number: 2021DZMEC-056-02).

2.4. Psoriasis area and severity index (PASI)

Photographs of the mouse skin lesions were taken against a uniform background every day before treatment. The degree of erythema, desquamation, and infiltration in mouse skin lesions was evaluated using the international PASI scoring standard, 0–4; three scores were added to obtain a total score range of 0–12, where the higher the score, the more serious the skin lesions.

2.5. Histological and immunostaining analyses

The dorsal skin was collected for histological and immunostaining analysis. Hematoxylin and eosin (HE) staining and immunohistochemical staining were performed on formalin-fixed, paraffin-embedded skin sections using antibodies against the following proteins: S1PR1 (Abcam, ab11424, 1:100); S1PR2 (Affinity, DF4921, 1:50); S1PR3 (Affinity, DF4869, 1:50); S1PR4 (Affinity, DF4872,

1:50); S1PR5 (NOVUS, NBP2–24712SS, 1:100); p-p65 (Affinity, AF2006, 1:100); p-Akt (Affinity, AF0016, 1:100); p-STAT3 (CST, 9145T, 1:100).

Images were collected under a microscope, and interactive image analysis (Image J 1.48) was performed to complete the measurement of epidermal skin thickness and immunohistochemical grayscale quantification on the backs of mice.

2.6. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) for detecting mRNA transcription of keratinogenic-related genes

From a fresh frozen skin tissue, total RNA was extracted using the TRIzol method, and RNA concentration and purity were determined. Using a reverse transcription kit, the RNA was reverse transcribed into cDNA. K1, K6, K16, K17, and involucrin (IVN) mRNA were amplified according to the qPCR kit instructions. The reaction system was as follows: cDNA (10 × dilution) 4 µl; Forward Primer (10 µM) 0.4 µl; Reverse Primer (10 µM) 0.4 µl; SYBR Green/Flourescein qPCR Master Mix (2 ×) 10 µl; H_2O 5.2 µl. The reaction conditions were: 50 °C for 2 min, 95 °C for 10 min; 95 °C for 30 s, and 60 °C for 30 s, for 40 cycles. The dissolution curve was drawn, and the final data were analyzed using the $2^{-\triangle\triangle Ct}$ method Primers were synthesized by Tsingke Biotechnology Co., Ltd.; the primer sequences are listed in Supplementary File Table 1.

2.7. Detection of keratinogenicity and NK-κB signaling pathway protein expression using western blotting

From a tissue sample, the total protein was extracted according to the instructions of the protein extraction kit, and the concentration was determined using the BCA kit. A total of 40 µg of proteins were separated using 12% gel electrophoresis. After transferring the membrane, the following primary antibodies against the indicated proteins were incubated overnight at 4 °C: GAPDH (1:1,000), K1 (1:1,000), K6 (1:300), K16 (1:1,000), K17 (1:1,000), IVN (1:1,000), p65 (1:2,000), p-p65 (1:1,000), Ikka (1:1,000), p-Ikka (1:1,000),
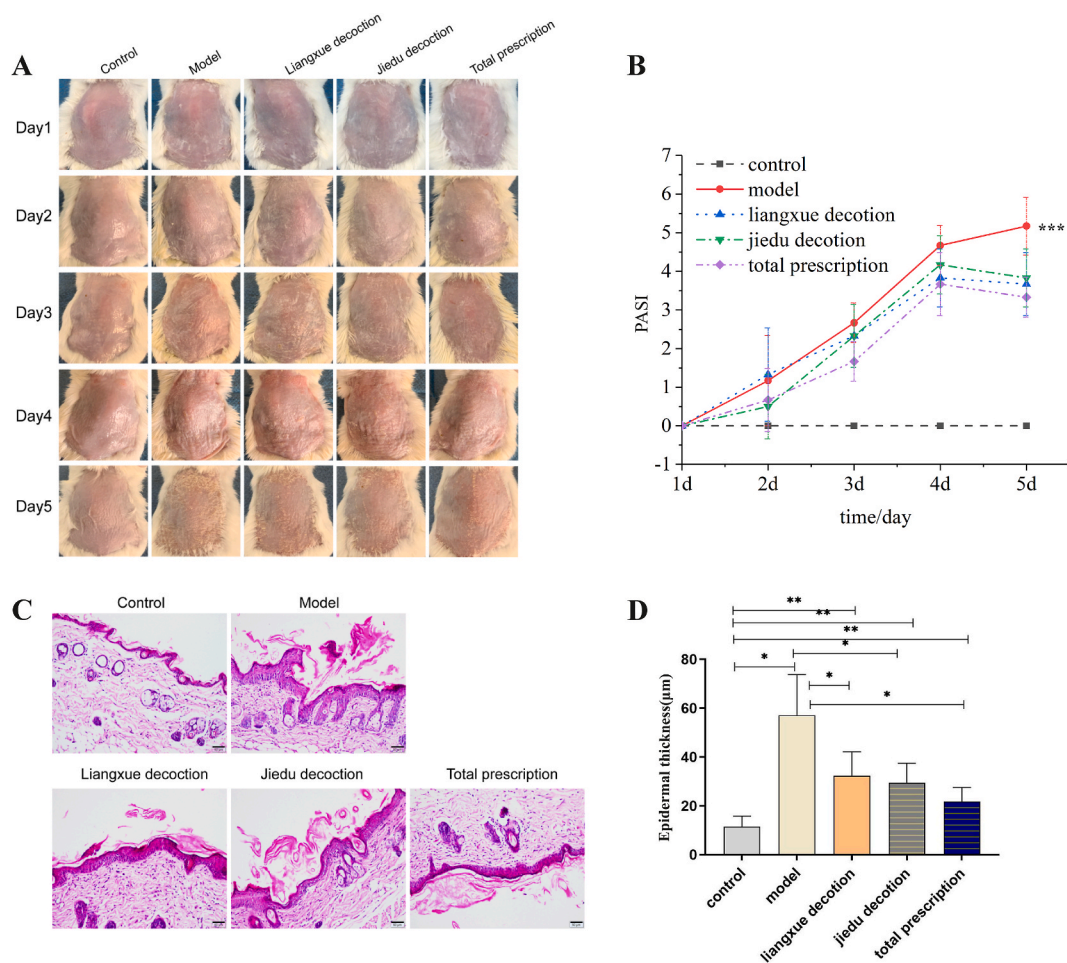

Fig. 1. Scores of skin lesions and disease severity of mice in each group. (A) Skin lesions on the backs of mice in each group; (B) comparison of PASI scores; (C) histopathological staining (× 200); and (D) comparison of epidermal thickness.

and IkBa (1:1,000). The membrane was washed five to six times with Tris buffered saline–Tween and incubated with horseradish peroxidase-labeled secondary antibody (1:50,000) at 37 °C for 2 h. The film was scanned after development, and the gray value was determined using BandScan 5.0.

2.8. EDetection of keratinocyte differentiation and Th17-related cytokine levels using enzyme-linked immunosorbent assay (ELISA)

Mouse serum and skin samples were collected and tested using ELISA detection technology. All reagents were thoroughly mixed before use, and blank, standard, and sample wells to be tested were respectively set up; three parallel experiments were performed for each group. Primary antibody incubation, washing, secondary antibody incubation, washing, and substrate color development were performed according to the instructions for each kit. After the reaction was terminated, the optical density (OD value) of each well was measured at a wavelength of 450 nm using a microplate reader. The tests used included the Mouse IL36G (interleukin-36 gamma) ELISA Kit (Finetest, EM1873), Mouse Interleukin-22 (IL-22) Enzyme-linked Immunoassay Kit (Buya-TEK, BYK1596), and Mouse Interleukin-17 (IL -17) Enzyme-linked Immunoassay Kit (Buya-TEK, BYK0827).

2.9. Determination of Th17 cell differentiation using flow cytometry

From the spleens of mice in each treatment group, peripheral blood mononuclear cells were isolated and placed in medium containing phorbol myristate acetate (50 ng/ml, BD), ionomycin (500 ng/ml, BD), and Golgi stop (1 g/ml, BD) for 4 h. Anti-CD4 and anti-IL-17 antibodies were used to detect the proportion of CD4$^+$IL-17$^+$ Th17 cells.

2.10. Statistical analysis

Excel statistical data, SPSS 20.0 statistical analysis and graphing, and measurement data were expressed as mean ± standard deviation ($\overline{x}$ ±s). Independence, normality, and homogeneity of variance were met, and one-way analysis-of-variance (ANOVA) was used to determine the differences between groups; otherwise, the non-parametric rank sum test (Kruskal–Wallis) was used. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Xiaoyin Jiedu Granules and the decomposed formula alleviate psoriasiform dermatitis induced by IMQ

To evaluate the effect of Xiaoyin Jiedu Granules on IMQ-induced PSO-like symptoms in BALB/c mice, we recorded the daily skin lesion status and calculated the PASI score (Fig. 1A and B); epidermal thickness measurements were performed in histopathological sections (Fig. 1C and D).

The mice in the control group showed no erythema, desquamation, or infiltrating changes, whereas those in the model group showed slight scales on days 3 to 4. On day 5, numerous layered scales appeared on the back skin, and the skin thickened. Compared with the model group, PSO-like skin lesions appeared later in the Liangxue, Jiedu, and total groups. Also, the degrees of erythema,
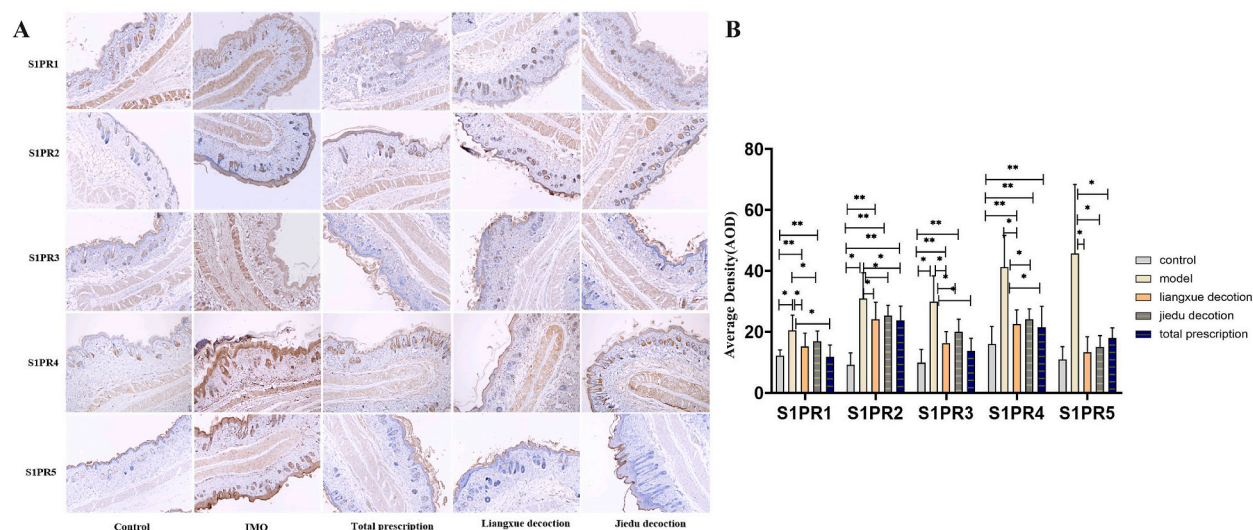

Fig. 2. Expression of S1PR1-5 in mouse skin lesions detected using immunohistochemistry (IHC). (A) IHC staining results (× 100); (B) comparison of the expression of S1PR1, S1PR2, S1PR3, S1PR4, and S1PR5 in skin tissues of mice in each group. IMQ, imiquimod; S1PR, sphingosine-1-phosphate receptor.

scales, and skin infiltration in the Liangxue, Jiedu, and total groups were lower than those in the model group. PASI scores showed that on day 5, the scores of the Liangxue, Jiedu, and total groups were significantly lower than those of the model group (P < 0.05). The thickness of the epidermis in the model group was significantly greater than that in the control group. Following treatment, the thickness of the epidermis of the three groups was significantly reduced, and the curative effect was the most obvious in the total group (P < 0.05).

3.2. Xiaoyin Jiedu Granules and the disassembled formula reduce the expression of S1PR1-5 in mouse skin tissue

To determine the effects of Xiaoyin Jiedu Granules on the expression of S1PRs, we detected the expression of S1PRs in the skin lesions of mice in each group. As shown in Fig. 2A, compared with that in the control group, the expression of S1PR1-5 in the model group was significantly increased (P < 0.05), and the distribution range was significantly wider. Both smooth muscle and endothelial cells in the model group expressed S1PR1-5.
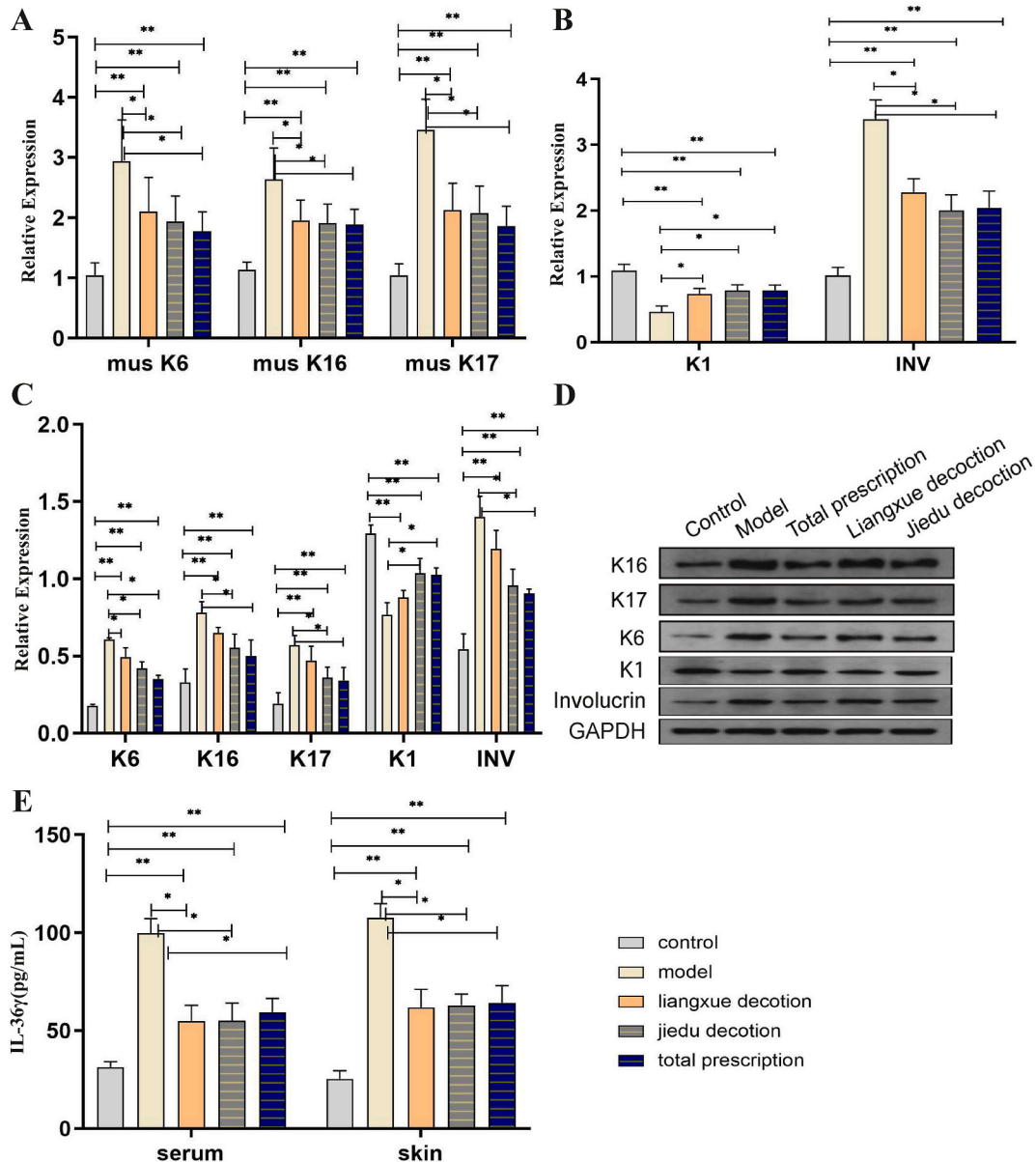

Fig. 3. Comparison of gene expression levels related to keratinocyte proliferation in mouse skin tissue of each group. (A) Quantitative polymerase chain reaction (qPCR) detection of expression of proliferation-related genes K6, K16, and K17; (B) qPCR detection of the expression of differentiation-related genes K1 and INV; see also Fig. S1; (C) western blotting of the relative expression levels of K6, K16, K17, K1, and INV; (D) western blotting results; (E) ELISA detection of IL-36γ content in mouse skin or serum.

Following treatment, the expression levels of S1PR1–5 in the skin lesions were significantly reduced, and the regulation effect in the total group was the most significant (P < 0.05, Fig. 2B).

3.3. Xiaoyin Jiedu Granules and the disassembled formula regulate the proliferation and differentiation of epidermal KCs

To determine the effect of Xiaoyin Jiedu Granules on the proliferation of skin KCs, we used qPCR to detect the mRNA transcription levels of keratin K1, K6, K16, K17, and IVN in the back skin tissue of PSO-induced model mice (Fig. 3A and B, Fig. S1). The results showed that, compared with those of the control group, the mRNA levels of keratin proteins IVN, K6, K16, and K17 in the model group were significantly increased (P < 0.05), whereas that of K1 significantly decreased (P < 0.05). Compared with those in the model group, each treatment group showed the converse expression changes; i.e., the severity of skin lesions was reduced (P < 0.05), among which the total group had the most significant effect. The protein levels of K1, K6, K16, K17, and IVN in the skin tissue of mice in each group were determined using western blotting (Fig. 3C and D).

These results were similar to those obtained using qPCR. Compared with the control group, the levels of IVN, K6, K16, and K17 in the model group increased significantly (P < 0.05), while that of K1 decreased significantly (P < 0.05). Compared with the model group, each treatment group showed reversed expression changes, i.e., the skin lesions were closer to those of the control group (P < 0.05), among which the Total group had the most significant effect.

We detected the level of the keratin inflammatory factor IL-36γ in the skin or serum of mice using ELISA (Fig. 3E). The results showed that, compared with the control group, the level of IL-36γ in the serum and back skin of the model group was significantly increased (P < 0.0001). Conversely, compared with the model group, the treatment significantly reduced the IL-36γ level in both the serum and skin (P < 0.05).
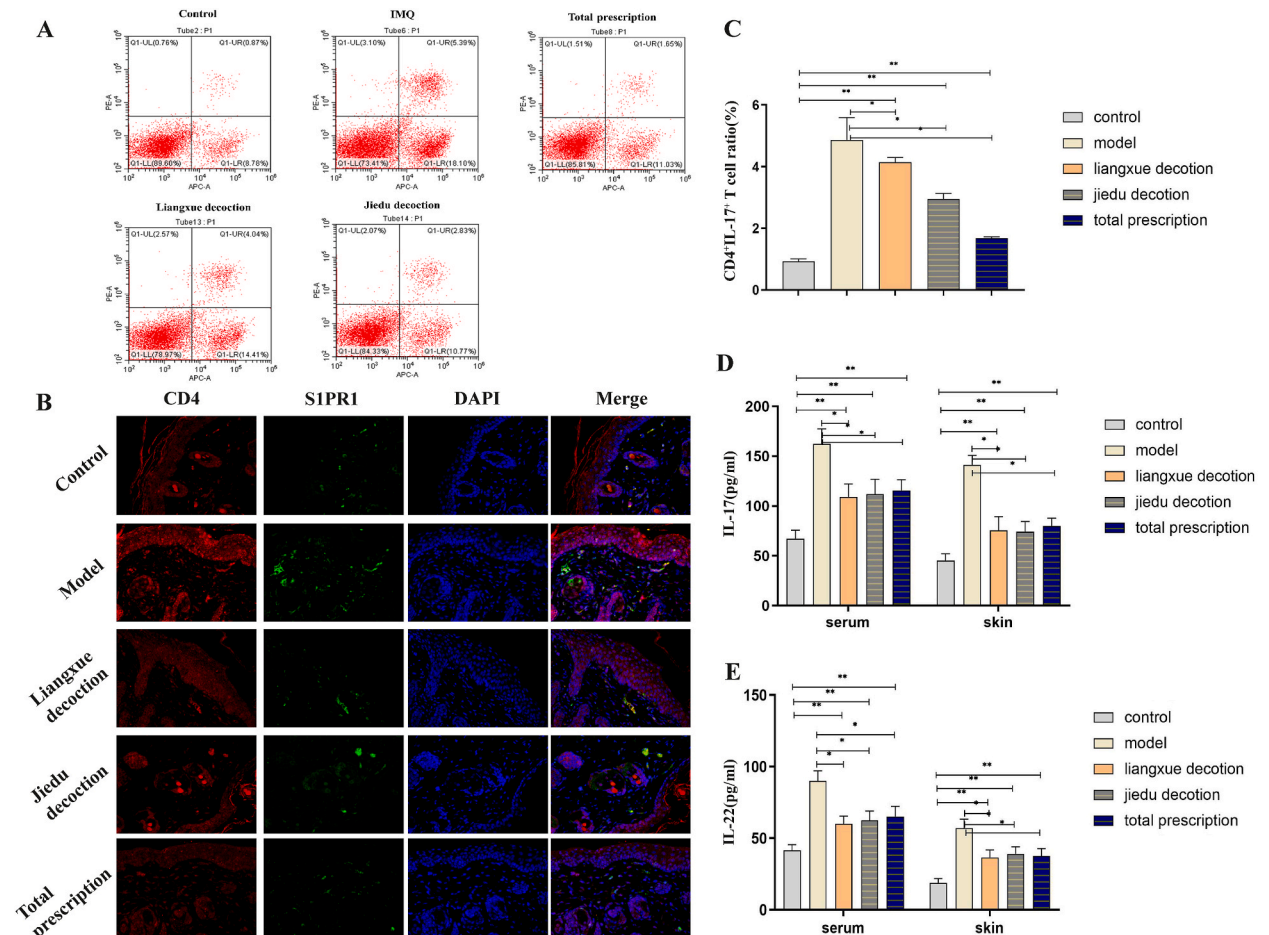

Fig. 4. Proportion of CD4⁺IL-17⁺T cells in the spleens of mice in each group and the expression of related inflammatory factors. (A) CD4⁺ and IL-17⁺ T cells detected using flow cytometry; (B) Immunofluorescence double staining of CD4⁺T cells and S1PR1 was performed in the psoriatic skin lesions of mice (× 400); (C) CD4⁺IL-17⁺ T cell ratio in each group; (D) IL-17 content in the skin and serum of each group; and (E) IL-22 content in the skin and serum of each group. IMQ, IMQ, imiquimod.

3.4. Xiaoyin Jiedu Granules and the disassembled formula reduce the differentiation of Th17 cells and reduce the secretion of inflammatory factors

The number of Th17 cells is correlated with the development and severity of PSO. We screened for CD4+IL-17+ T cells using flow cytometry (Fig. 4A) and immunohistochemistry (Fig. 4B) and showed that, compared with that of the control group, the proportion of CD4 and IL-17 co-positive spleen T cells in the model group was significantly increased (Fig. 4C, P < 0.05). Moreover, the treatment inhibited the proportion of CD4 and IL-17 co-positive T cells in mouse spleen induced by IMQ cream, with the inhibitory effect of the total group being the most significant.

Th17 cells mainly produce IL-23, IL-22, TNF-α, IL-17, and IL-6. We detected the levels of IL-17 and IL-22 in the peripheral blood and skin tissue of mice in each group using ELISA (Fig. 4D and E) and showed that, compared with those in the control group, the levels of
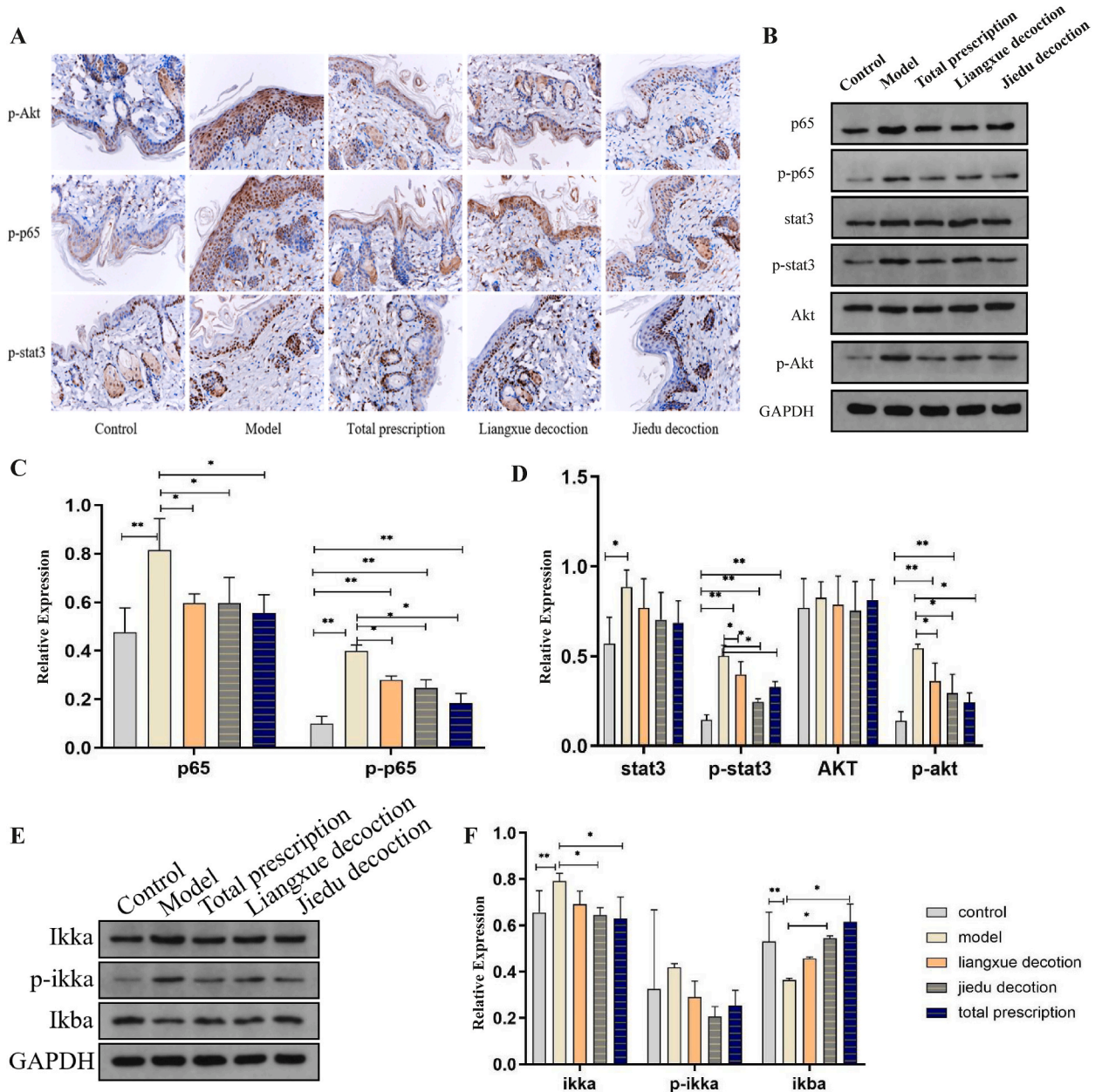

Fig. 5. Expression of inflammation-related signaling pathway proteins in mice in each group. (A) IHC detection of p-Akt, p-p65, and p-STAT3 in mouse skin lesions (× 400), see also Fig. S2; (B) western blotting of Akt, p65, STAT3, and p-Akt, p-p65, p-STAT3 in skin tissue; (C) relative expression of p65, p-p65; (D) relative expression of STAT3, p-STAT3, Akt, and p-Akt; (E) western blotting of Ikka, p-Ikka, and Ikba levels in skin tissue, See also Fig. S3; (F) relative expression levels of Ikka, p-Ikka, and Ikba.

IL-17 and IL-22 in the model group were significantly upregulated (P < 0.05). Conversely, the levels of IL-17 and IL-22 in the skin tissue and serum of the mice in each treatment group were lower than those in the model group (P < 0.05); however, there was no significant difference among the treatment groups (P > 0.05).

3.5. Xiaoyin Jiedu Granules and the disassembled formulas mitigate the progression of PSO by regulating the NF-κB pathway

Inflammatory pathways associated with PSO include NF-κB, STAT3, and PI3K/Akt pathways. We detected the mRNA expression of p-Akt, p-p65, and p-STAT3 in mouse skin lesions using IHC (Fig. 5A) and the protein expression of Akt, p65, STAT3, and p-Akt, p-p65, p-STAT3 using western blotting (Fig. 5B, Fig. S2). The results showed that p65 and p-p65 were significantly increased in the model group, whereas their expression levels were significantly reduced in the treatment groups (P < 0.05, Fig. 5C). STAT3 expression in the model group was significantly higher than that in the control group (P < 0.05) and was slightly decreased in the treatment groups; however, this difference did not reach significance (P > 0.05, Fig. 5D). The level of p-STAT3 was significantly increased in the model group and was significantly lower in the total and Jiedu groups (P < 0.05, Fig. 5D). There was no change in the Akt level in all groups (P > 0.05, Fig. 5D); the p-Akt level was significantly increased in the model group and significantly decreased in the treatment groups (P < 0.05, Fig. 5D). These results implicate p65 and p-p65 in PSO and suggest that Xiaoyin Jiedu Granules and the disassembled formulas can effectively inhibit their expression.

Based on these results, we detected the expression of Ikka, p-Ikka, and Ikba, which belong to the NF-κB pathway along with p65 (Fig. 5E, Fig. S3). The results showed that, compared with that of the control group, the expression of Ikka and p-Ikka in the model group was significantly upregulated, and compared with that in the model group, the expression of Ikka in the mouse skin tissue of the total and Jiedu groups was downregulated (Fig. 5F, P < 0.05). Compared with that in the control group, the expression of Ikba in the model group was significantly downregulated, and compared with that in the model group, the expression of Ikba in the mouse skin tissue of the total and Jiedu groups was upregulated (P < 0.05).

4. Discussion

PSO is a common chronic inflammatory skin disease with typical skin lesions characterized by hyperproliferation, dysregulated epidermal KC differentiation, and immune cell infiltration [1]. The pathogenesis of PSO is believed to arise from local skin infiltration by immune effector cells and inflammatory factors, which leads to abnormal proliferation and incomplete differentiation of KCs.

In this study, the appearance and histology of IMQ-treated mice were similar to those of human psoriatic lesions, showing signs of skin redness, thickening, and desquamation. S1PR1–5 in the skin of PSO inflammation-model mice showed different degrees of high expression. Although at different levels, the expression of S1PRs changed synchronously with the severity of PSO. This suggests that the downstream signals regulated by S1PRs and S1P may lead to the abnormal proliferation of psoriatic KCs. Previous studies have confirmed that S1P/S1PRs can interfere with the transport and distribution of immune cells: S1PR1 and S1PR3 induce the migration of T lymphocytes during the immune process [11], S1PR4 is involved in the migration of neutrophils from blood to tissues [12], and S1PR2 can increase vascular permeability [13]. Jeon et al. found that inhibiting the synthesis of S1P or antagonizing the function of S1PRs can effectively promote the differentiation of human KCs and improve PSO-like dermatitis in mice [14]. Shin et al. proposed that the mechanism by which inhibiting the S1P/S1PR axis improves the inflammation associated with PSO involves the blockage of Th17 differentiation [15].

The imbalance of CD4$^+$ T cell subsets is a key factor in the pathogenesis of PSO [16]. Activated Th17 cells facilitate the activation of innate immunity, synergizing with B cells. Conversely, cytokines, such as IL-17 and IL-22, secreted by Th17 cells play an essential role in the pathogenesis of PSO. IL-17 has the ability to upregulate the expression of proinflammatory cytokines in KCs [17], while directly cooperating with other inflammatory factors to exert a proinflammatory effect. IL-22 regulates cells in skin tissue, especially epithelial tissue, and promotes KCs to secrete MMP1, MMP3, and other proteases that accelerate skin proliferation, but inhibits KC differentiation-related genes, leading to epidermal hyperplasia and parakeratosis in PSO lesions [18]. IL-36γ is a newly discovered molecular marker of PSO expressed in KCs. Evidence suggests that IL-36γ may amplify innate and acquired immunity, and its function is associated with KC differentiation and maturation [19]. Furthermore, its expression is positively correlated with the severity of PSO [20,21]. In the present study, Th17 differentiation and the expression of related inflammatory factors were positively correlated with the expression of S1PRs, consistent with previous findings, suggesting that the S1P/S1PR/Th17 axis influences the progression of PSO.

Following the induction of PSO via IMQ, the expression of S1PRs increased, which significantly increased the proportion of Th17 cell polarization, and the expression of Th17-related cytokines IL-17, IL-22, and IL-36γ. Compared with those in the PI3K-AKT-mTOR signaling pathway and STAT3 signaling pathway, the PSO model showed more significant changes in the NF-κB pathway, which was allayed by our treatment intervention. The expression of p65 and p-p65 in the skin of mice with PSO was significantly increased, which was mitigated by our treatment. The Th17/IL17 signaling pathway can promote the activation of IκB kinase (IKK) by recruiting TRAF6, leading to the release of NF-κB and increasing the transcription of NF-κB target genes [22]. In this study, the expression of Ikka and p-Ikka in mice with PSO was significantly increased, whereas that of the NF-κB inhibitor IkBα was decreased; the expression of each index in the treatment groups was closer to that of the control group. Therefore, we speculate that the Xiaoyin Jiedu Granules regulate the expression of S1PRs, inhibit the activation and proliferation of Th17 cells, the reduce the secretion of related cytokines and activation of the NF-κB signaling pathway, thereby reducing the inflammation associated with PSO.

KCs in PSO are characterized by excessive proliferation and incomplete differentiation as the basic pathological features. Keratin K6, K16, and K17 [23,24] are associated with KC hyperproliferation and are overexpressed in psoriasis lesions; K1 and IVN, markers of early and late differentiation, respectively, are expressed at low and high levels in PSO [25]. Compared with that in normal skin, the

expression of IVN in PSO lesions occurs earlier and at significantly higher levels, which is closely associated with the abnormal regulation of proliferation and differentiation of KCs in patients with PSO. Moreover, IVN up-regulation is related to the stimulation of inflammatory factors such as IL-17A, TNF-α, and IFN-y [26].

The means by which Xiaoyin Jiedu Granules affect disease progression through S1PRs remains unclear. In the present study, oral administration of Xiaoyin Jiedu Granules and the disassembled formula effectively alleviated the PSO-like skin lesions induced by IMQ in mice, with both the macroscopic PASI score and the microscopic measurement of epidermal thickness supporting this finding. The total group showed more significant results compared with those of the other two split groups. Drug intervention in each treatment group reduced the high expression of S1PRs in the epidermis of mice with PSO. Following treatment, the secretion of keratin K6, K16, K17, and involucrin was inhibited, the expression of K1 was increased, and the accumulation of IL-36γ in the circulation was reduced. This suggests that Xiaoyin Jiedu Granules can reduce the expression of S1PRs, inhibit the function of the S1P/S1PR axis, inhibit Th17 and its downstream pathways, and relieve inflammation. Xiaoyin Jiedu Granules can also inhibit the proliferation of KCs and promote differentiation to improve PSO. Compared with the Liangxue and Jiedu groups, the total group showed better results in all aspects. In most cases, the data obtained from the Liangxue and Jiedu groups were not significantly different.

The Jiedu group showed a greater decrease in CD4$^+$Th17 cell differentiation compared to that of the Liangxue group. Moreover, the keratin level in the skin tissue of the Jiedu group approached that of healthy mice, whereas the Liangxue group did not exhibit a similar effect. Therefore, the drug effects of these two groups may overlap; however, the unique underlying mechanism of action of each remains to be determined. The total group synergistically achieved superior curative effects with regard to PSO.

Together, our findings validated the expression of S1PRs in skin lesions of mice with PSO-like dermatitis and established the correlation between Xiaoyin Jiedu Granules treatment and the downregulation of S1PRs, Th17 cell differentiation (a major mediator of inflammation in PSO), and keratinization (a major pathological manifestation in PSO). Our findings shed light on the major inflammatory pathways and biological mechanisms involved in disease progression. Moreover, this study laid a theoretical foundation for evaluating the specific roles of each S1PR in disease pathogenesis, such as through generation of receptor-deficient cell models by small-interfering RNA-mediated knockdown of each receptor subtype. Nevertheless, this study has some limitations. First, the biological mechanism underlying the effect of Xiaoyin Jiedu Granules on PSO warrants further pharmacologic investigation. Furthermore, the failure to accurately measure the circulating and tissue S1P levels and its association with Th17 differentiation renders it difficult to determine how the S1P/S1PR axis influences PSO progression. In the future, we intend to further explore the role of S1P in PSO, to facilitate its clinical application.

5. Conclusion

Xiaoyin Jiedu Granules can effectively inhibit IMQ-induced psoriatic dermatitis in mice, and its mechanism of reducing inflammation may be associated with inhibiting the S1P/S1PR-Th17/KC inflammatory response axis and reducing IL-22 and IL-17. The results of treatment in the Jiedu group was superior to that of the Liangxue group with regard to inhibiting Th17 cell differentiation and keratin expression, whereas combination treatment can synergistically enhance the curative effect.

Author contribution statement

Zi Wang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Guangzhong Zhang: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.;
Haomin Zhang: Performed the experiments; Analyzed and interpreted the data.
Lingling Li: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data..

Funding statement

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19109.

References

[1] C.E.M. Griffiths, A.W. Armstrong, J.E. Gudjonsson, J.N.W.N. Barker, Psoriasis, Lancet 397 (10281) (2021) 1301–1315, https://doi.org/10.1016/s0140-6736(20)32549-6.
[2] K. Chen, G. Wang, H. Jin, J. Xu, X. Zhu, M. Zheng, et al., Clinic characteristics of psoriasis in China: a nationwide survey in over 12000 patients, Oncotarget 8 (28) (2017) 46381–46389, https://doi.org/10.18632/oncotarget.18453.
[3] C.E.M. Griffiths, J.M. van der Walt, D.M. Ashcroft, C. Flohr, L. Naldi, T. Nijsten, et al., The global state of psoriasis disease epidemiology: a workshop report, Br. J. Dermatol. 177 (1) (2017), https://doi.org/10.1111/bjd.15610 e4–e7.
[4] K. Bocheńska, M. Gabig-Cimińska, Unbalanced sphingolipid metabolism and its implications for the pathogenesis of psoriasis, Molecules 25 (5) (2020) 1130, https://doi.org/10.3390/molecules25051130.
[5] B. Burger, R.N. Sagiorato, I. Cavenaghi, H.G. Rodrigues, Abnormalities of sphingolipids metabolic pathways in the pathogenesis of psoriasis, Metabolites 13 (2) (2023), https://doi.org/10.3390/metabo13020291, 13484–13350.
[6] N. Gray, M.-M. Limberg, A.-U. Bräuer, U. Raap, Novel functions of S1P in chronic itchy and inflammatory skin diseases, J. Eur. Acad. Dermatol. Venereol. 36 (3) (2022) 365–372, https://doi.org/10.1111/jdv.17764.
[7] A. Checa, N. Xu, D.G. Sar, J.Z. Haeggström, M. Ståhle, C.E. Wheelock, Circulating levels of sphingosine-1-phosphate are elevated in severe, but not mild psoriasis and are unresponsive to anti-TNF-α treatment, Sci. Rep. 5 (2015), 12017, https://doi.org/10.1038/srep12017.
[8] S.N. Syed, R. Raue, A. Weigert, A. von Knethen, B. Brüne, Macrophage S1PR1 signaling alters angiogenesis and lymphangiogenesis during skin inflammation, Cells 8 (8) (2019) 785, https://doi.org/10.3390/cells8080785.
[9] A.A.L. Baeyens, S.R. Schwab, Finding a way out: S1P signaling and immune cell migration, Annu. Rev. Immunol. 38 (2020) 759–784, https://doi.org/10.1146/annurev-immunol-081519-083952.
[10] X. Chen, R. Zhang, X. Duan, M. Xue, T. Qu, L. Li, Effectiveness of Xiaoyin Jiedu granules in the treatment of psoriasis vulgaris in patients with blood-heat symptom patterns in terms of Traditional Chinese Medicine, J. Tradit. Chin. Med. 40 (5) (2020) 863–869, https://doi.org/10.19852/j.cnki.jtcm.2020.05.017.
[11] M. Aoki, H. Aoki, R. Ramanathan, N.C. Hait, K. Takabe, Sphingosine-1-phosphate signaling in immune cells and inflammation: roles and therapeutic potential, Mediat. Inflamm. (2016), 8606878, https://doi.org/10.1155/2016/8606878, 2016.
[12] M.L. Allende, M. Bektas, B.G. Lee, E. Bonifacino, J. Kang, G. Tuymetova, et al., Sphingosine-1-phosphate lyase deficiency produces a pro-inflammatory response while impairing neutrophil trafficking, J. Biol. Chem. 286 (9) (2011) 7348–7358, https://doi.org/10.1074/jbc.m110.171819.
[13] H. Okamoto, N. Takuwa, T. Yokomizo, N. Sugimoto, S. Sakurada, H. Shigematsu, et al., Inhibitory regulation of Rac activation, membrane ruffling, and cell migration by the G protein-coupled sphingosine-1-phosphate receptor EDG5 but not EDG1 or EDG3, Mol. Cell Biol. 20 (24) (2000) 9247–9261, https://doi.org/10.1128/mcb.20.24.9247-9261.2000.
[14] S. Jeon, J. Song, D. Lee, G.-T. Kim, S.-H. Park, D.-Y. Shin, et al., Inhibition of sphingosine 1-phosphate lyase activates human keratinocyte differentiation and attenuates psoriasis in mice, J. Lipid Res. 61 (1) (2020) 20–32, https://doi.org/10.1194/jlr.ra119000254.
[15] S.-H. Shin, K.-A. Cho, S. Hahn, Y. Lee, Y.-H. Kim, S.-Y. Woo, et al., Inhibiting sphingosine kinase 2 derived-sphingosine-1-phosphate ameliorates psoriasis-like skin disease via blocking Th17 differentiation of naïve CD4 T lymphocytes in mice, Acta Derm. Venereol. 99 (6) (2019) 594–601, https://doi.org/10.2340/00015555-3160.
[16] L. van der Fits, S. Mourits, J.S.A. Voerman, M. Kant, L. Boon, J.D. Laman, et al., Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis, J. Immunol. 182 (9) (2009) 5836–5845, https://doi.org/10.4049/jimmunol.0802999.
[17] J.R. Chan, W. Blumenschein, E. Murphy, C. Diveu, M. Wiekowski, S. Abbondanzo, et al., IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis, J. Exp. Med. 203 (12) (2006) 2577–2587, https://doi.org/10.1084/jem.20060244.
[18] L.M. Perusina, Y. Lin, J. Fang, W. Zou, T. Frankel, Biological and pathological activities of interleukin-22, J. Mol. Med. 94 (5) (2016) 523–534, https://doi.org/10.1007/s00109-016-1391-6.
[19] J.J. Pyo, S. Ahn, S.H. Jin, S. An, E. Lee, J. Choi, et al., Keratinocyte-derived IL-36γ plays a role in hydroquinone-induced chemical leukoderma through inhibition of melanogenesis in human epidermal melanocytes, Arch. Toxicol. 93 (8) (2019) 2307–2320, https://doi.org/10.1007/s00204-019-02506-6.
[20] S. Madonna, G. Girolomoni, C.A. Dinarello, C. Albanesi, The significance of IL-36 hyperactivation and IL-36R targeting in psoriasis, Int. J. Mol. Sci. 20 (13) (2019) 3318, 10.3390%2Fijms20133318.
[21] L. Zhou, V. Todorovic, Interleukin-36: structure, signaling and function, Adv. Exp. Med. Biol. 21 (2021) 21191–21210, https://doi.org/10.1007/5584_2020_488.
[22] N.C. Brembilla, L. Senra, W.-H. Boehncke, The IL-17 family of cytokines in psoriasis: IL-17A and beyond, Front. Immunol. 9 (2018) 1682, https://doi.org/10.3389/fimmu.2018.01682.
[23] X. Zhang, M. Yin, L.-J. Zhang, Keratin 6, 16 and 17-critical barrier alarmin molecules in skin wounds and psoriasis, Cells 8 (8) (2019) 807, https://doi.org/10.3390/cells8080807.
[24] L. Jin, G. Wang, Keratin 17: a critical player in the pathogenesis of psoriasis, Med. Res. Rev. 34 (2) (2014) 438–454, https://doi.org/10.1002/med.21291.
[25] H.J. Bovenschen, M.M. Seyger, P.C. Van de Kerkhof, Plaque psoriasis vs. atopic dermatitis and lichen planus: a comparison for lesional T-cell subsets, epidermal proliferation and differentiation, Br. J. Dermatol. 153 (1) (2005) 72–78, https://doi.org/10.1111/j.1365-2133.2005.06538.x.
[26] J.-Q. Chen, X.-Y. Man, W. Li, J. Zhou, L. Landeck, S.-Q. Cai, et al., Regulation of involucrin in psoriatic epidermal keratinocytes: the roles of ERK1/2 and GSK-3β, Cell Biochem. Biophys. 66 (3) (2013) 523–528, https://doi.org/10.1007/s12013-012-9499-y.