**Spatholobus Suberectus Column Extract Suppresses Dendritic Cell Maturation and has Therapeutic Potential for Psoriasis**

Yan Wang¹,², Jingxia Zhao¹,², Tingting Di¹,², Xiaolong Xu¹,², Mingxing Wang¹,², Yujiao Meng¹,², Xiangjiang Xie¹,², Zhitong Ruan¹,², Lu Zhang¹,², Yan Lin¹,², Xin Liu¹,², Ning Wang¹,² and Ping Li¹,²*

¹Beijing Key Laboratory of Clinic and Basic Research with Traditional Chinese Medicine on Psoriasis, Beijing Hospital of Traditional Chinese Medicine, Affiliated with Capital Medical University, Beijing, China
²Beijing Institute of Traditional Chinese Medicine, Beijing, China

**Abstract**

*Spatholobus Suberectus* Column Extract (SSCE) is a natural product from the plant *Spatholobus suberectus* Dunn, a kind of Traditional Chinese Medicine, which is widely used to invigorate the circulation of blood and replenish blood, has antitumor and anticoagulant properties and improves hematopoiesis. This study evaluated the clinical effects of *Spatholobus Suberectus* Column Extract (SSCE) in an Imiquimod (IMQ) induced psoriasis mouse model and investigated its role in regulating the differentiation and maturation of Dendritic Cells (DCs). BALB/c mice were used to establish the animal model for psoriasis-like skin lesion; SSCE at 12 mg/kg (high), 6 mg/kg (medium) and 3 mg/kg (low) respectively, were intragastrically administered. Psoriasis Area and Severity Index (PASI) was used to evaluate the skin lesions. Histological changes, the thickness of epidermis and the quantity of CD11c+ DCs in skin lesion and spleens were measured. *In vitro* experiments, bone marrow cells of mice were obtained, and CD11c+ cells were isolated. DCs with a mature state in differentiation and function were identified by flow cytometry. The influence of DCs on proliferation of allogenic lymphocytes was analyzed with CCK-8. SSCE treatment alleviated psoriasis-like skin with the decreased Psoriasis Area and Severity Index (PASI) score and obviously reduced the vertical thickness of epidermis. Besides, SSCE treatment decreased the quantity of CD11c+ DCs in skin lesion and spleens. Furthermore, SSCE reduced R848-induced murine bone marrow-derived DC maturation, characterized by reduced expression of CD80/86 and inhibited the alloproliferation of T cells.

*Corresponding author:* Ping Li, Beijing Key Laboratory of Clinic and Basic Research with Traditional Chinese Medicine on Psoriasis, Beijing Hospital of Traditional Chinese Medicine, Affiliated with Capital Medical University, Beijing, China

**Keywords:** Dendritic cells; Psoriasis; *Spatholobus suberectus* column extract

**Introduction**

Psoriasis is a chronic inflammatory disorder of the skin affecting approximately 3% of the world’s population. It is the most common chronic inflammatory skin disease characterized by epidermal hyperplasia, scaling and erythematous plaque formation [1]. Although the pathogenesis of psoriasis is not fully elucidated, it is widely accepted that cellular infiltration of T cells, Dendritic Cells (DCs) and neutrophils is important in the pathogenesis of psoriasis by provoking inflammation [2].

DCs are key players in the immune mechanisms surrounding psoriasis as well as other autoimmune diseases [3]. DC-induced cytokine production subsequently stimulated T-cell activity [4]. A cross-talk between DCs and T cells is thought to be responsible for the disease development [5]. Recently, the clearance of psoriasis using targeted immunotherapies demonstrates the important role of DCs in the pathogenesis of psoriasis.

*Spatholobus Suberectus* Column Extract (SSCE) is a natural product from the plant *S. suberectus* Stem, a kind of Traditional Chinese Medicine, which is widely used to invigorate the circulation of blood and replenish blood, has antitumor and anticoagulant properties, and improves hematopoiesis [6-8]. Current research has shown its potential value in immunomodulation and the management of autoimmune diseases. Considering the pharmacological effects of *S. suberectus* spatholobus stem, it was speculated that these substances could block the pathological changes brought about by psoriasis in various aspects [9,10].

This study determined whether SSCE exhibited antigorscapic activity in a mouse model. It was the first study to evaluate the effects of SSCE on DCs *in vitro* and *in vivo*.

**Materials and Methods**

**Extraction and isolation of *Spatholobus suberectus* Dunn**

The whole plants of *Spatholobus suberectus* Dunn (0.1 kg) were bought from Beijing Hospital of Chinese Medicine. The dried and milled whole plants of *Spatholobus suberectus* Dunn (0.1 kg) were soaked with 500 mL 80% ethanol solution overnight, and extracted with water under reflux for thrice. The extracts were filtered by 180 mesh sieve filtration, concentrated to thick paste then vacuum drying to get crude extract *Spatholobus suberectus* Dunn. The crude extract was dissolved with deionized water under reflux for thrice. The extracts were filtrated by 180 mesh sieve filtration, concentrated to thick paste then vacuum dying to get crude extract *Spatholobus suberectus* Dunn. The crude extract was dissolved with deionized water and centrifugated, the supernatant was placed in the polyamide column to elute and collect the, SSCE.

**Animals and grouping**

Male BALB/c mice (18 to 20g) were bought from Beijing Huafukang Biotechnology Stock Company Ltd (License No: SCXK (Jing) 20090007). All animal experiments were performed in accordance with the guidelines for the care and use of Laboratory Animals.
(Beijing Institute of Traditional Chinese Medicine). This animal study was approved by the Animal Care and Scientific Committee of Beijing Institute of Traditional Chinese Medicine. Mouse were randomized into four groups: (1) high-dose SSCE group (SSCE-H) with animals intragastrically administered SSCE dissolved in normal saline at a dose of 12 mg/kg/day for 6 days; (2) medium-dose SSCE group (SSCE-M), SSCE at 6 mg/kg/day for 6 days; (3) low-dose SSCE group (SSCE-L), SSCE at 3 mg/kg/day for 6 days [11]; (4) Model group (Model), normal saline of the same volume as in SSCE-treatment groups for 6 days; (5) methotrexate (Shanghai Pharmaceutical Co., Ltd. Shanghai) group (MTX), with animals receiving methotrexate dissolved in normal saline at 1 mg/kg/day for 6 days. Mice treated with Vaseline (lanolin Feitian Petrochemical Co., Ltd., Hebei) and intragastrically normal saline were used as controls (Ctrl). After 6-days continuous treatment, the mice were executed through cervical vertebra dislocation and their skin was collected. The skin was separated into two parts for Hematoxylin and Eosin (H&E) staining after preparing paraffin sections and immunohistochemistry.

Scoring of skin inflammation severity

To score the severity of back skin inflammation, an objective scoring system was developed based on the clinical Psoriasis Area and Severity Index (PASI). Erythema, thickness, and scaling were scored independently on a scale from 0 to 4: 0, none; 1, slight; 2, moderate; 3 marked; 4 very marked. The score for each group was averaged, and trend lines were generated to observe the changes in mouse skin lesions [12].

Histology

Tissue slices (5 μm) were cut from the paraffin sections and stained with H&E for pathological observation by light microscopy. Epidermal thickness was accurately measured using the Image Pro Plus software. (Leeds Precision Instruments, MN, USA).

Immunohistochemistry

Paraffin-embedded sections were dewaxed for 1 h with xylene and placed into a sodium citrate solution for 10 min at 95°C in a water bath for antigen retrieval. Then, the sections were fixed in 3% H2O2 for 10 min at room temperature, and the slides were incubated overnight at 4°C with primary antibodies against CD3 (1:200, Abcam, USA), PCNA (1:800, Abcam, USA) and Ki67 (1:200, Abcam, USA). This was followed by incubation for 30 min with secondary antibodies. Frozen sections were fixed in 3% H2O2 for 10 min at room temperature. Slides were incubated overnight at 4°C with primary antibodies against CD11c (1:200, Abcam, USA), followed by incubation for 30 min with secondary antibodies. Slides were observed with Olympus DP20 (Japan) and semi-quantification of the staining was performed independently by two researchers using IPP6.0. Each index was presented as integrated Optical Density (OD).

Cell culture

C57 mice were executed by cervical dislocation, immersed in 75% alcohol for 2-3 min. The skin was cut off to take out and bony ends were cut off. RPMI1640 (HyClone, USA) was extracted using a syringe, and the bone marrow cells were washed. Tris-NH Cl buffer was added to remove the red cells. Then, the cells were counted and seeded into a six-well plate with flat bottom at a density of 0.5-1x10^6/mL with RPMI 1640 medium containing granulocyte macrophage-colony-stimulating factor and interleukin-4 (Pepro Tech, USA), 100 ng/mL each and cultured at 37°C with 5% CO2. On Day 6, CD11c+ cells were purified using a magnetic bead method (Stem Cell, USA), and CD11c expression levels on the cell surface were detected by flow cytometry (BD, USA) to identify DCs. Cells were counted by Handheld Automated Cell Counter (Millipore, USA).

Cell treatment

On Day 6, the cells were divided into five groups the control group received no additional treatment, the model group was treated with 1 μg/mL R848 for 24h and the other three groups were pretreated with SSCE at the concentrations of 15, 7.5 and 3.75 μg/mL for 3h and then treated with 1 μg/mL R848 for 24h.

Flow cytometric analysis

Inguinal spleen's samples from each group were minced through a 70-mm mesh to obtain single cell suspensions. Fluorescence-conjugated monoclonal Antibodies (mAbs) recognizing CD11c, CD80, CD86, MHC-II and their respective isotype controls were obtained (eBioscience, USA). Cell surface staining was performed by incubating 5x10^5 cells in 100 μL of staining buffer containing optimal concentrations of mAb specific for a receptor or an Ig isotype-matched control for 20 min. After incubation, the cells were washed with PBS (Amresco, USA). Samples were analyzed using a flow cytometer (BD, USA).

Mixed homogenous lymphocyte reaction

Cells of all groups were treated with 25 μg/mL mitomycin C, incubated at 37°C for 45 min, and washed with phosphate-buffered saline three times. Then, the cells were suspended in an RPMI 1640 medium and seeded in round-bottomed 96-well plates at the density of 1x10^4/well. Homogeneous lymphocytes (4x10^4) were added into each well to make up a final volume of 200 μL. Three replicates were performed for each group. The cells were cultured for 5 days, and then 10 μL of Cell Counting Kit-8 (CCK-8, Dojindo, Japan) was added into each well for further incubation of 3h. The OD was measured using a microplate reader, and the average value of three wells was calculated.

Statistical analysis

All quantitative data were expressed as the mean ± standard deviation. Comparisons between two groups were assessed using the Student’s t-test, and comparisons among three or more groups were evaluated via one-way analysis of variance followed by post hoc test with SPSS software 15.0 (IL, USA). P values less than 0.05 were considered to indicate statistical significance.

Results

SSCE effectively attenuated psoriatic lesions and alleviated the histopathologic changes in IMQ-induced psoriasis-like mouse model

To value whether SSCE was beneficial in IMQ-induced psoriasis-like mouse model, mice were treated with SSCE 12, 6 and 3 mg/kg. After 6-days treatment with drugs, psoriatic lesions characterized by erythema, thickening and scaling were observed in the IMQ group, while SSCE significantly alleviated the clinical changes in the IMQ-treated mice (Figure 1A). Moreover, the disease severity was assessed by a clinical scoring system. It was found that SSCE significantly reduced the PASI scores after 6-days treatment (Figure 1B).
SSCE reduced the infiltration of T cells and neutrophils in IMQ-induced psoriasis-like mouse lesions

Cellular infiltration of neutrophils, keratinocytes and T cells in lesions plays vital roles in the pathogenesis of psoriasis. Immunohistochemistry using antibody against CD3 and Ki67 showed the abundant infiltration of T cells and keratinocytes in dermis when compared with the IMQ group (Figure 2A-B).

SSCE decreased the number of CD11c+ DCs in IMQ-induced psoriasis-like mouse lesions

Immunofluorescence Assay (IFA) was used to detect the number of CD11c+ DCs in the dorsal skin of IMQ-induced mice. Compared with the normal control group, the number of CD11c+ DCs significantly increased in the model group but significantly reduced in the SSCE and MTX group in comparison to the model group (Figure 2C). The number of CD11c+ DCs in spleens was detected using flow cytometer, and the results were similar to those of skin lesion (Figure 2D).

Effect of SSCE on cell viability in vitro

Cell viability was determined by CCK-8. Bone marrow derived dendritic cells were incubated overnight in 96-well plates with flat bottom at a density of 5×10^3 cells per well. After 24h, the cells were treated with different concentrations (250 μg/ml, 125 μg/ml, 62.5 μg/ml, 31.25 μg/ml, 15 μg/ml and 7.5 μg/ml) of SSCE for another 20h. Then 10 μL of CCK-8 (5%) was added into each well for further incubation of 4h. The OD was measured at 570 nm using a microplate reader (Bio-Rad, USA) (Figure 3).

Effects of SSCE on the phenotypic and functional maturation of DCs

As shown in figure 4A and 4B, a phenotypic analysis showed that 95% of the cells expressed CD11c after purifying with magnetic bead method. R848 was used to induce maturation of DCs with a resultant enhanced surface expression of CD80 and CD86. SSCE treatment inhibited the R848 effect on the maturity markers in CD80 and CD86. These results indicated that SSCE inhibited DC maturation.

A main characteristic of mature DCs is their capacity of inducing allogeneic T-cell proliferation. To further investigate the effect of SSCE on the maturation of DCs, the allogeneic mixed lymphocyte reaction was examined. It was found that R848 increased CD4+ T-cell proliferation, while SSCE inhibited the capacity of allogeneic T-cell proliferation (Figure 4C).

Discussion

This study demonstrated that SSCE alleviated psoriasis-like skin with the decreased PASI score and obviously reduced the vertical thickness of epidermis. Furthermore, SSCE prevented the maturation of murine Bone Marrow Dendritic Cells (BMDCs), characterized by reduced levels of CD80/86, and reduced all proliferation of T cells. These results suggested that SSCE regulated DC maturation, and that it might serve as a novel therapeutic agent for psoriasis.
Although SSCE has been reported to be an anticancer agent [13,14], the effect of SSCE in psoriasis and DCs has not yet been determined. This study found that SSCE treatment alleviated IMQ-induced psoriasis mice model, inhibited BMDC maturation and suppressed proliferation of allogeneic T cells. These results suggested that SSCE could inhibit the maturation and activation of DCs and might be of potential value in treating some autoimmune diseases.

Imiquimod (IMQ) is a TLR7/8 agonist, and IMQ-induced mouse model is a classic model of psoriasis [15]. To investigate the effect of SSCE on psoriasis, the effect of SSCE on IMQ-induced psoriasis mice model was evaluated. It was observed that SSCE effectively attenuated psoriatic lesions, alleviated the histopathologic changes, reduced the infiltration of T cells and neutrophils in psoriasis-like mouse lesions, and reduced the number of CD11c+ DCs in mouse lesions and spleens.

DCs are thought to be the initiators of some autoimmune diseases such as psoriasis. Maturation and activation of DCs are key steps in triggering the priming of autoreactive peripheral T cells, which then drive the development of inflammatory responses [16-19]. The effect of SSCE on DCs maturation was evaluated in R848-induced BMDCs. It was observed that SSCE treatment in R848-induced BMDCs resulted in a significant reduction in the expression of DC maturity markers and inhibited the capacity of allogeneic T-cell proliferation.

Conclusion

In summary, the present study showed that SSCE inhibited DCs maturation by reducing the expression of maturity markers, suppressed the ability of allogeneic T-cell proliferation, and effectively improved the disease condition of mice with psoriasis. The present data not only clarified a new cellular mechanism for the anti-inflammatory and immunosuppressive effects of SSCE, but also indicated the therapeutic potential for psoriasis.

Disclosures

The authors have no financial conflicts of interest.

Acknowledgment

The study was supported by National Natural Science Foundation of China (No. 81403410).

References
