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Formula PSORI-CM01 inhibits the inflammatory cytokine and chemokine release in keratinocytes via NF-κB expression

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ABSTRACT

Psoriasis is a common chronic inflammatory disease in which T-helper 1(Th1) and T-helper 17(Th17) cells play an important role in its pathology. Formula PSORI-CM01 was a novel formulated Chinese medicine used for psoriasis therapy. It had been demonstrated previously that PSORI-CM01 and serum contained Formula PSORI-CM01 (PCM01CS) could improve psoriasis by inhibiting the epithelial hyperplasia, how PSORI-CM01 affects inflammatory cytokine and chemokine in dermis is still unknown. In this study we found PSORI-CM01 pre-treated 3 days before IMQ painting could ameliorated IMQ-induced mice skin lesion as PASI score was apparently reduced. Th1 related cytokine IFN-γ and Th17 related cytokine IL-17/IL-22 was used to induce inflammatory models on human keratinocyte cell line HaCaT in vitro, respectively. PCM01CS significantly reduced IFN-γ induced mRNA expression of IL-6, IL-12 and CXCL-10, reduced IL-6 and CXCL-10 release into HaCaT supernatant. 20 ng/ml IL-17/IL-22 co-stimulation significantly upregulated expression of IL-6, IL-8 and CCL20 mRNA expression in HaCaT cells, PCM01CS significantly inhibit these cytokines expression both in mRNA and in protein levels. Finally, PCM01CS could obviously inhibit nuclear NF-κB p65 expression which activated by IFN-γ and IL-17/IL-22 stimulation. Thus, our new findings reveal that Formula PSORI-CM01 may possess therapeutic action on psoriasis by inhibiting inflammatory within skin environments.

1. Introduction

Psoriasis is a common immune cell mediated chronic inflammatory skin disease that affects 2–3% of the population in the world. Although the pathogenesis of psoriasis is largely unknown, infiltration of leukocytes into the dermis and its release of cytokines, chemokines, and growth factors play an important role in the maintenance and recurrence of psoriasis.

IFN-γ which most produced by Type 1 helper T cells (Th1) and IL-17 or IL-22which produced by Th17 cells, is considered to be the main inflammatory factors in psoriasis, and was found that these inflammatory factors increased not only in the peripheral blood of patients with psoriasis, but also in the skin lesions. Anti-cytokine therapies might be the most effective methods currently available for treating psoriasis [1].

Chinese herbal medicines are combined into formulae, and used to treat psoriasis vulgaris for many years either by topical or oral administration. Now more and more traditional Chinese medicine formulae have been proved to be effective by randomized, double-blind, placebo-controlled clinical trial, and their mechanism of action is studied by using the currently accepted animal models or cell models [2–5].

Formula PSORI-CM01 is optimized from an inter-hospital preparation Yinxieling tablet which originated by Xuan Guowei, a nationally famous dermatologist in Guangdong Provincial Hospital of Chinese medicine [6]. PSORI-CM01 was composed of seven herbs including *Radix PaeoniasRubra*, *Rhizoma Curcumas*, *Saracandraglabra*, *Rhizoma Smilacis Glabras*, *FructusMume*, *Radix arnebias*, and *Radix Glycyrrhizas*. It has been reported that Formula PSORI-CM01 could be used to treat moderate to severe psoriasis vulgaris and without significant side-effects [7–8]. Moreover, animal experiments on guinea pig were also demonstrated [9], that Formula PSORI-CM01 dramatically inhibited the ear skin erythema stimulated by the Propranolol. Our previous

Abbreviations: PSORI-CM01, formula PSORI-CM01; PCM01CS, serum contained PSORI-CM01; IMQ, imiquimod; PASI, Psoriasis Area Severity Index; IFN-γ, interferon-γ; HE, Hematoxylin and eosin; IL-6, interleukin 6; IL-8, interleukin 8; IL-12, interleukin 12; IL-17, interleukin17; IL-22, interleukin22; IL-23, interleukin23; CXCL-1, the chemokine (C-X-C motif) ligand 1; CXCL-10, the chemokine (C-X-C motif) ligand 10; CXCL-16, the chemokine (C-X-C motif) ligand 16; CCL20, chemokine (C-C motif) ligand 20; TGFA, transforming growth factor α; TNFA, tumor necrosis factor α; NF-κB, nuclear factor κB; STAT3, signal transducer and activator of transcription 3.

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study showed PSORI-CM01 could inhibit epidermal hyperplasia in imiquimod (IMQ)-induced mouse psoriasis-form model and reduces keratinocyte proliferation in vitro [10]. In the present study, we focused on the anti-inflammatory effects of Formula PSORI-CM01 in eliminating cytokines and chemokines release within inflammated keratinocyte micro-environments after stimulated by IFN-γ, or IL17/IL22.

2. Materials and methods

2.1. Reagents

Tissue Freezing Medium was from SAKURA (Alphen aan den Rijn, NL), α-MEM medium was from GIBCO (Beijing, China). Fetal bovine serum was from GIBCO (NY, USA), Trizol, RT reagent Kit and SYBR® Premix Ex Taq™ II was from TAKARA (Dalian, China), RIPA buffer and GAPDH rabbit mAb were from Cell Signaling Technology (Beverly, MA, USA), BCA Protein Assay Kit was from Thermo Scientific (DE, USA),

NF-κB fluorescence labeling antibody, PCNA, rabbit polyclonal IgG was from Santa Cruz (TX, USA), Goat anti-rabbit IgG-HRP was from CALBIOCHEM (Darmstadt, Germany), IL17, IL22, IFNγ (protech, Israel), High-glucose DMEM, FBS, antibiotics, and trypsin-EDTA were obtained from Invitrogen.

2.2. The formula PSORI-CM01

Formula PSORI-CM01 is mainly composed of *Radix PaeoniaeRubra* (chi shao), *Sarcandraglabra* (jiujiecha), *RhizomaSmilacisGlabrae* (tufuling), and other four botanicals. Formulae PSORI-CM01 has the functions of invigorating blood circulation, tonifying *Qi* and removing heat from the blood. PSORI-CM01 granules were provided by Guangdong Provincial Hospital of Chinese medicine.

Identification and quantitative characterization of PSORI-CM01 by liquid chromatography coupled with an LTQ Orbitrap MS has been finished [11]. 3-O-caffeoylqauinic acid, paeoniflorin, liquiritin, astilbin, engeletin, liquiritigenin and glycyhhizic acid were the main active components of PSORI-CM01.

2.3. Preparation of drug contained serum of Formula PSORI-CM01(PCM01CS)

20 SD rats, body weight 220–250 g, were purchased from Guangdong Medical Laboratory Animal Center. The animal experimental protocol were reviewed and approved by the Institutional Animal Care and Use Committee of Guangzhou University of Chinese Medicine. All experimental procedure performed were conducted in accordance with institutional guidelines for the care and use of laboratory animals in research.

Rats were randomly divided into 2 groups: control and PSORI-CM01 group, after 3 days acclimatization. Based on clinical dosage of PSORI-CM01, the dosage for rats (8.8 g/kg) was 10 times the daily adult dosage. Medicine was given through intragastric administration while equivalent volumes of normal saline were given for the control group. After 5 days administration, the rats were anesthetized with 10% chloral hydrate solution at 1 h after the last feed, and then sacrificed by rapid exsanguinations via the abdominal aorta. Blood was drawn into sterile test tubes and centrifuged at 3500 r/min for 15 min. The serum was collected, denatured at 56 °C for 30 min, filtrated through a 0.22 μm filter to remove bacteria, and stored at −70 °C. The final concentration of control or drug serum in cell medium is 12% based on our previous study.

2.4. Mouse model of psoriasis

BALB/C mice (male, 18 to 20 g, 3 week old) were purchased from Guangdong Provincial Animal Laboratory Centre, after 3 days acclimatization, mice were divided into control, IMQ model, and PSORI-CM01 group. Except control group, mice in other 2 groups would receive a daily topical painting of 60 mg cream containing 5% Imiquimod (IMQ, SiChuanMingXin) on the mice shaved back for continuous 6 days. 1.14 g/kg PSORI-CM01 granules was intragastric administrated 3 days before IMQ painting, and lasted until the end of IMQ. The skin lesion severity was monitored and graded using a modified human scoring system Psoriasis Area Severity Index (PASI), the score could reflex severity of inflammation in skin lesion. To calculate the PASI score, the psoriasis plaques found on mice back skin region will first grade for their combined redness, thickness (induration), and scaling. The severity of the plaques on back skin is graded on a 0 to 4 scale, with 0 meaning no involvement and 4 meaning severe involvement. The 3 components of the PASI score: red, skin thickening (induration), as well as scaling was also classified with 0–4 rating, with 0 meaning no involvement and 4 meaning severe involvement. Skin samples were also used for HE staining and immunohistochemical staining was used to detect the expression of CD45 in each group.

2.5. Cell line and Cultures

The human keratinocyte (HaCaT) cell line was purchased from the Storage Centre of Wuhan University, and was cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), penicillin, and streptomycin at 37 °C in 5% CO_2 incubator.

2.6. Inflammatory model of HaCaT cells

The cells were serially passaged at 60–70% confluence, and the experiments were conducted at passage three using subconfluent cells (60–80% confluence) in the proliferative phase, unless otherwise specified. For total RNA extraction and Western blot analysis, keratinocytes were cultured in 6 and 12-well tissue culture plates, respectively. PSORI-CM01-contained serum or control serum would be added together with stimulator at different concentration and different time. The final concentration of IFN-γ was 20 U/ml, and final concentration of IL-17/IL22 was 20 ng/ml respectively.

2.7. Total RNA extraction and real-time PCR

Total RNA was extracted with TRIZOL reagent according to the manual. Spectrophotometer (Thermo Scientific/NANO drop 2000, DE, USA) as used to determine the concentration and purity of total RNA at A260nm/A280nm. cDNA synthesis was performed with a PrimeScript ™ RT reagent kit according to the manufacturer's instruction. The volume of qPCR reaction system was 20 μl, in which the template was 2 μl, the final concentrations of primers were 200 nM, and each sample was detected in duplicate. The reaction condition was determined in accordance with SYBR Premix Ex Taq™ reagent instructions, then the melting curves were analyzed for production specificity in a Real Time PCR system (ABI, 7500, NY, USA). Primers are shown in detail in Table 1.

2.8. Western blot analysis

Sub-confluent keratinocytes were incubated with various stimulants, and lysateds were obtained by lysing the cells in RIPA buffer (Cell Signaling Technology). The total protein concentrations were determined using the Precisong Red Advanced Protein Assay Kit

Table 1
Primers applied in the experiments.

Gene	Primer (F/R)
Il-6	ACTCACCTCTTCAGAACGAATTG; CCATCTTTGGAAGGTTCAGGTTG
IL-12A	CCTTGCACTTCTGAAGAGATTGA; ACAGGGCCATCATAAAAGAGGT
CXCL10	GTGGCATTCAAGGAGTACCTC; TGATGGCCTTCGATTCTGGATT
IL-8	TTTTGCCAAGGAGTGCTAAAGA; AACCCTCTGCACCCAGTTTTC
CCL20	TGCTGTACCAAGAGTTTGCTC; CGCACACAGACAACTTTTTCTTT
GAPDH	GGTCTCCTCTGACTTCAACA; AGCCAAATTCGTTGTCATAC