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Research paper

Curcumin shows excellent therapeutic effect on psoriasis in mouse model

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ABSTRACT

Curcumin is an active herbal ingredient possessing surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activity. Recently, it has been reported to exhibit inhibitory activity on potassium channel subtype Kv1.3. As Kv1.3 channels are mainly expressed in T cells and play a key role in psoriasis, the effects of curcumin were investigated on inflammatory factors secretion in T cells and psoriasis developed in keratin (K) 14-vascular endothelial growth factor (VEGF) transgenic mouse model. Results showed that, 10 μM of curcumin significantly inhibited secretion of inflammatory factors including interleukin (IL)-17,IL-22, IFN-γ, IL-2, IL-8 and TNF-α in T cells by 30–60% *in vitro*. Notably, more than 50% of T cells proliferation was inhibited by application of 100 μM curcumin. Compared with severe psoriatic symptoms observed in the negative control mice, all psoriasis indexes including ear redness, weight, thickness and lymph node weight were significantly improved by oral application of curcumin in treatment mouse group. Histological examination indicated that curcumin had anti-inflammatory function in the experimental animals. More than 50% level of inflammatory factors including TNF-α, IFN-γ, IL-2, IL-12, IL-22 and IL-23 in mouse serum was decreased by curcumin treatment as well as cyclosporine. Compared with renal fibrosis observed in the mouse group treated by cyclosporine, no obvious side effect in mouse kidney was found after treated by curcumin. Taken together, curcumin, with high efficacy and safety, has a great potential to treat psoriasis.

1. Introduction

A diversity of physiological and pathophysiological function of potassium channel has been well described with some remarkable progress achieved, since the first cloning of Kv channel was successfully done more than 20 years ago [1]. K^+ channels are ubiquitous in a variety of cells, neurons, cardiac myocytes, skeletal muscles, smooth muscles, pancreatic β-cells, lymphocytes and tumor cells. Kv1.3 channel has been demonstrated to be expressed predominately in T cells, effector memory T lymphocytes (TEM) and notably up-regulated in activated TEM cells, where in the number of channel is found to be increased from approximately 250 to 1500 channels per cell [2,3]. The activation of Kv1.3, controlling the efflux of K^+ out the cells, provides persistent electrical signal and a rise in intracellular Ca^{2+} concentration, which alters the proliferation and activation of T cells [4,5]. Many scientists believe that blockers of the Kv1.3 channel could be potential therapeutic interests to treat autoimmune diseases. A sea anemone toxin ShK, with 35 amino acid residues, is reported to show a high affinity to Kv1.3 with an IC_{50} value of 10 pM [6]. A synthetic analog of ShK, ShK-186, is reportedly supposed to be a therapeutic for autoimmune diseases and has already been scheduled to phase-1 clinical in 2011 [7].

Millions of people have been suffering from psoriasis, a kind of serious autoimmune inflammatory disease usually occurred in the skin and joints. Intraregional T-lymphocytes trigger primed basal stem keratinocytes to proliferate and perpetuate the disease

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process of psoriasis [8–11]. Transgenic, spontaneously mutated or chemically induced mouse models were used for understanding the pathogenesis of psoriasis and subsequently screening for anti-psoriatic compounds. Those ideal mouse models are supposed to show the same symptom to human, such as histological, cellular and molecular features of psoriatic skin. A mouse cDNA encoding $VEGF_{164}$ was used to generate K14-VEGF transgenic mice imitate the psoriatic of human [12]. These transgenic mice resulting inflammatory skin condition with profound cellular and molecular features of psoriasis, including the characteristic vascular changes and epidermal alterations.

Curcumin (diferuloylmethane), isolated from a rhizomatous herbaceous perennial plant *Curcumalonga (Linn)* (turmeric), has been used as a traditional Chinese Medicine for a very long period to treat abdominal pain, as well as for liver disorders, diabetic wounds, rheumatism, anorexia, and menstrual difficulties [13,14]. In recent years, curcumin has been well studied and some of its pharmacological properties have been described. For instance, it is reported to interact with multiple targets and possess activities on decreasing cell proliferation and enhancing apoptosis of tumor cells. Importantly, curcumin shows potential effects against the development of pancreatic cancer, skin cancer, breast cancer and colorectal cancer in animal models [15]. It has been regarded as a complementary therapy to the treatment of psoriasis in clinical, but the mechanism is still unknown [16–19].

Besides those functions stated above, curcumin reduces the Kv currents in rabbit coronary arterial smooth muscle cells where it acts as a potent blocker of Kv channels [20]. In addition, curcumin also inhibits Kv1.3 currents in Jurkat T cells and T_{EM} [21,22]. In this study, curcumin was found to inhibit currents of Kv1.3 channel and the proliferation of T cells as well. Moreover, curcumin showed a potent effect on a psoriasis model of 14-VEGF transgenic mice.

2. Methods

2.1. Reagents

Curcumin, purchased from Sigma–Aldrich(St Louix, MO), was prepared as a 10 mM stock solution in dimethyl sulfoxide (DMSO). Antibodies to human CD3 and CD28 were from BD Biosciences (San Jose, CA). All other chemicals used were purchased from Sigma–Aldrich.

2.2. Transient transfection and cell culture

Two plasmids encoding Kv1.3 and green fluorescent protein were transfected to human embryonic kidney 293 (HEK293) cells [23]. Cells were grown under standard tissue culture conditions (5% CO_2, 37 °C) in DMEM supplemented with 10% FBS. After 3 h, HEK293 cells were washed with fresh medium.

2.3. Patch-clamp recording on human Kv1.3 channel

HEK293 cells with GFP fluorescence were selected for patch-clamp recordings after 36–72 h of transfection. K^+ currents were recorded using an internal solution containing the followings (in mM): KCl 140, $MgCl_2$ 2.5, HEPES 10, and EGTA 11 (pH 7.2). The external bathing solution contained the followings (in mM): NaCl 150, KCl 5, $CaCl_2$ 25, $MgCl_2$ 12, HEPES 10, and D-glucose 10 (pH 7.2). Whole-cell patch-clamp recordings were carried out at room temperature (20–25 °C) using an EPC-10 amplifier (HEKA, Germany) [24].

2.4. Human T cell isolation

T cells from peripheral blood mononuclear cells (PBMCs) of healthy volunteers were isolated by negative magnetic depletion using biotin-conjugated CD14, CD15, CD16, CD19, CD34, CD56, CD123 and CD235a (Human Pan T Cells Isolation Kit; Miltenyi Biotec). The PBMCs were separated using Ficoll-Hypaque density gradient centrifugation for 15 min at 3000 rpm following the manufacturer's protocol. The purity of T_{EM} cells was assessed by flow cytometry to be more than 95%. Then the T cells were maintained in RPMI 1640 medium (Thermo) supplemented with 10% FBS (Thermo), penicillin (100 U/ml) and streptomycin (100 μg/ml) in 5% CO_2 at 37 °C on 96-well plates (105 cells/well) for 1 h. Approval to conduct these studies was obtained from the ethics committee of the Institutional Review Board of the Kunming Institute of Zoology, Chinese Academy of Sciences (2013-116). All participants were provided written informed consent for the use of blood samples.

2.5. Cytokine secretion by human CD3+ T cells

Curcumin and Cyclosporin A (Cs A) were diluted in PBS and then added 1 h prior to bead stimulation. The isolated CD3+ T cells were activated using anti-CD3+/CD28 + dynabeads (Invitrogen) at a T cell: bead ratio of 1:1 in 200 μL of RPMI medium in 96-well plates, in triplicate. After a 16-h activation, cells were counted and supernatant were analyzed for determining the concentration of cytokines (hTNF-α, hIL-2, hIL-8, hIL-17, hIL-22 and hIFN-γ) using ELISA (R&D, USA) following the manufacturer's instructions.

2.6. Effects of curcumin in a mouse model of psoriatic

Three months old keratin 14-VEGF transgenic mice with a psoriatic form phenotype were selected for further experiment (♀18, ♂18) [12]. Mice were divided into three group, then were treated with saline, curcumin or Cs A. Curcumin (40 mg/kg) or Cs A (40 mmol/kg) were administered orally to mice every 24 h and a psoriatic-form phenotype of ears was photographed every 5 days during the treatment. Four parameters of psoriasis including ear

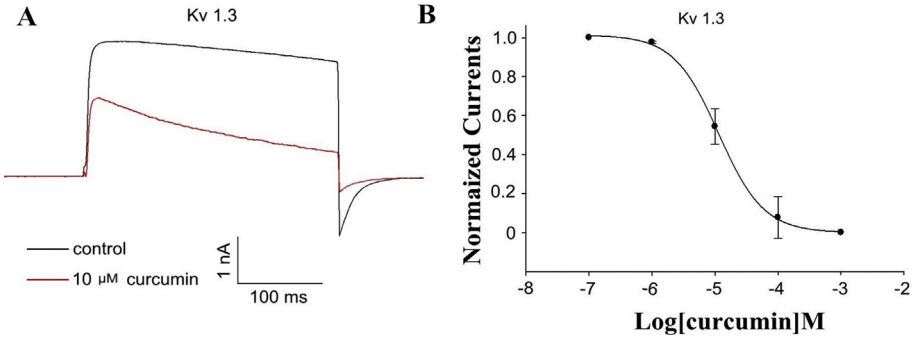

Fig. 1. Effect of curcumin on Kv1.3 channel expressed in HEK293 cells. (A) Currents were elicited by a 300 ms depolarization of +10 mV from a holding potential of −80 mV. The red line was the currents elicited after added 10 μM curcumin. (B) Concentration-response curve for curcumin inhibition of Kv1.3 currents in HEK293T cells (n = 5).