International Immunopharmacology 21 (2014) 375–382

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

The molecular mechanism of curcumol on inducing cell growth arrest and apoptosis in Jurkat cells, a model of CD4$^+$ T cells

Heng Wang [a,b], Yong Wang [a,*], Xiaoji Jiang [a], Zhizhong Wang [a], Bing Zhong [b], Yongfei Fang [b,*]

[a] Department of Traditional Chinese Medicine, Southwest Hospital, Third Military Medical University, Chongqing 400038, PR China
[b] Department of Rheumatology, Southwest Hospital, Third Military Medical University, Chongqing 400038, PR China

ARTICLE INFO

Article history:
Received 26 September 2013
Received in revised form 16 May 2014
Accepted 19 May 2014
Available online 28 May 2014

Keywords:
Rheumatoid arthritis
Curcumol
Jurkat cells
Jak3–STAT
Mechanism

ABSTRACT

CD4$^+$ T cells in rheumatoid arthritis (RA) express growth signaling pathway in association with deregulated growth and resistance to apoptosis. The janus kinase (Jak) 3 and signal transducer and activator of transcription (STAT) pathway play a critical role in interleukin-2 (IL-2)-induced CD4$^+$ T cell proliferation. The present study aimed to explore the anti-cell proliferation mechanism of curcumol, a pure monomer extracted from Chinese medical plant *Rhizoma curcumae*. Cell proliferation was determined using WST-1 assay after curcumol treatment. The cell cycle distribution and Bcl-2 protein expression were assessed by flow cytometry. The cellular morphology of apoptosis was evaluated by Hoechst 33258 staining. The expressions of phosphorylated-Jak3 (p-Jak3), p-STAT3, and p-STAT5a following IL-2 stimulation were determined by western blot analysis. The Electrophoretic Mobility Shift Assay was used to detect the DNA binding activities of transcription factors STAT3 and STAT5. The study results showed that curcumol could inhibit the IL-2-induced Jurkat cell proliferation in a concentration- and time-dependent manner in vitro. Curcumol could cause cell cycle arrest at the S phase, induce cell apoptosis, and inhibit the expression of Bcl-2 in a dose-dependent manner. Curcumol at 50 μg/mL and Jak3 inhibitor ZM39923 could inhibit the phosphorylation of Jak3 and STAT5a. In conclusion, the underlying mechanism of curcumol on suppressing CD4$^+$ T cell proliferation and inducing apoptosis might partly be mediated by inhibition of Jak3–STAT5-related molecular activities and Bcl-2 expression, respectively; further studies are required in vivo to test the use of curcumol as a promising therapeutic option for RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that is characterized by hyperplasia of synovial membrane and infiltration of macrophages and lymphocytes into the joints, mediated partly by chemokines and cytokines [1]. The precise pathogenesis of RA has not been completely elucidated, but infiltration of a large number of CD4$^+$ T lymphocytes and hyperplasia of synoviocytes in the synovial membrane are considered to induce and exacerbate synovitis of RA [2,3]. Apoptosis is the process of programmed cell death that may occur in multicellular organisms to maintain tissue homeostasis. Resistance to apoptosis and elevated expression of B cell lymphoma-2 (Bcl-2) in CD4$^+$ T cells from patients with RA are linked to excessive infiltration of lymphocytes [4]. Transfer of synovial-infiltrating T cell derived from patients with RA into mice with severe combined immunodeficiency disease (SCID) could cause inflammatory arthritis. Moreover, elimination or prevention of T cells by CD4 monoclonal antibody could ameliorate RA disease activity [5,6]. Hence, all these evidences suggest that CD4$^+$ T cells play key roles in initiating and sustaining synovial inflammation in RA.

At present, there is a lack of novel and safer drugs for effective treatment of RA. Therapeutic plant extracts or herbal formulations can be an alternative and complementary source for modern medicine in the development of new drugs [7]. Curcumol, a major monomeric chemical substance, was isolated from *Rhizoma curcumae* in 1965, and its absolute stereo-structure was determined in 1984 [8]. Only few pharmacological investigations have been reported to date on curcumol, possibly due to its low water solubility. Curcumol inhibits the development of hepatic fibrosis. The underlying mechanism is believed through downregulation of transforming growth factor-β1 and cytochrome P450a in HSC-T6 cells [9]. Emerging evidence indicates that curcumol may have the potential to prevent and treat tumors. Curcumol has been reported to cause dose-dependent cell death via a caspase-independent mitochondrial pathway in ASTC-a-1 cells [10]. The proliferation was inhibited and apoptosis of nasopharyngeal carcinoma cell line, CNE-2, was induced by curcumol, possibly through downregulating the nuclear factor (NF-κB) protein level [11]. Our previous study has demonstrated that curcumol could suppress the proliferation of fibroblast-like synoviocytes and DNA synthesis induced by platelet-derived growth factor-BB through janus kinase (Jak) 2 and signal transducer and activator of transcription (STAT) signal

* Corresponding authors.
E-mail addresses: wangyongjhy@126.com (Y. Wang), fangyongfei@qq.com (Y. Fang).

http://dx.doi.org/10.1016/j.intimp.2014.05.021
1567-5769/

pathway [12]. However, considering the importance of $CD4^+$ T cells in RA, the effect and molecular mechanism of curcumol on $CD4^+$ T cell proliferation and apoptosis have not been documented yet.

Jurkat cells, expressing CD4 molecules on the surface, are frequently chosen as the biological model of $CD4^+$ T cells in vitro in RA research, because their cell phenotypes and certain functions are similar to those of normal T cells [13,14]. The aim of the present study was to explore the effect and mechanism of curcumol on cell proliferation and apoptosis regarding Jak3–STAT signal transduction induced by (interleukin) IL-2. Jurkat cells were treated with curcumol in vitro and the cytotoxic effects of curcumol on cell viability; cell cycle distribution; apoptosis; STAT3 and STAT5 activities; and protein expressions of phosphorylated Jak (p-Jak) 3, STAT3, and STAT5a were observed. Results showed that the underlying mechanism of curcumol on suppressing $CD4^+$ T cell proliferation and inducing apoptosis might partly be mediated through the downregulation of Jak3–STAT5 related molecular activities and Bcl-2 expression, respectively.

2. Materials and methods

2.1. Reagents and antibodies

Curcumol ($C_{15}H_{24}O_2$) was separated from *Zedoary Turmeric* Oil (vapor distillated from *R. curcumae*) by combining crystal precipitation in lower temperature (0–5 °C) with anhydrous ethanol recrystallization and characterized by chromatography with curcumol reference substance (obtained from National Institute for the Control of Pharmaceutical and Biological Products, batch number: CUL081007T, used for assay). The purity of curcumol was greater than 98% by peak normalization method using high performance liquid chromatography with ultraviolet detection. Working solutions were prepared by dissolving the compound in dimethylsulfoxide (DMSO) prior to the experiments. Anti-Bcl-2-fluorescein isothiocyanate (FITC) monoclonal antibody was obtained from BioLegend (CA, USA). Recombinant human interleukin-2 (rhIL-2) was purchased from PeproTech (NJ, USA). 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1) cell proliferation and cytotoxicity assay kit were obtained from KeyGEN (Nanjing, China). Rabbit anti-phospho-Jak3 (Tyr980/981) and anti-phospho-STAT3 (Tyr705) monoclonal antibodies were purchased from Cell Signaling Technology (MA, USA). Rabbit anti-phospho-STAT5a (S780) polyclonal antibody was obtained from BioVision (CA, USA). LightShift Chemiluminescent Electrophoretic Mobility Shift Assay (EMSA) Kit was purchased from Pierce (IL, USA). Jak3 inhibitor (ZM39923 hydrochloride) was purchased from Sigma-Aldrich (MO, USA).

2.2. Cell culture

Jurkat cell line was a gift from Dr. San Youan of Southwest Hospital. Suspended cells were cultured in RPMI 1640 medium (HyClone, USA) supplemented with 10% fetal bovine serum (Chengdu, China), 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin and maintained at 37 °C in a 5% CO_2 atmosphere. After three passages, 5×10^4 viable cells were added to each well of a 96-well cell culture plate for cell proliferation assay, and 1×10^6 cells were added to each well of a six-well culture plate for other detections. Curcumol was first dissolved in absolute DMSO and then diluted with the culture medium to a series of concentrations. The final concentration of absolute DMSO should not exceed 0.5% of the total culture volume. A solvent control group (0.5% absolute DMSO without curcumol) and IL-2 group (50 ng/mL of IL-2 for 30 min) were applied in cell proliferation. For Western blot and EMSA, cells were randomly divided into several groups: normal control group and IL-2 group, and the remaining groups were treated with Jak3 inhibitor ZM39923 50 μmol/L or 50 μg/mL curcumol preinduced by IL-2 for 30 min. Then, the plate was incubated at 37 °C in a 5% CO_2 atmosphere for 24 h.

The ratio of expressing CD4 molecules in all Jurkat cells was (56.20 ± 6.18) % in three independent experiments. Therefore, suspended Jurkat cells, expressing CD4 molecules on the surface, were chosen as the biological model of $CD4^+$ T cells in vitro.

2.3. Cell proliferation assay

Jurkat cells were seeded (5×10^4 cells/well) into flat-bottom 96-well culture plates in triplicate and activated with rhIL-2. Cells were treated with various concentrations (6.25, 12.5, 25, and 50 μg/mL) of curcumol for 24 h and 50 μg/mL of curcumol at different time periods (3, 6, 12, and 24 h). The effect of curcumol on cell viability was measured by WST-1 assay according to the manufacturer's instructions [15].

2.4. Cell cycle determination

Cell cycle distribution was analyzed by flow cytometry (FCM). After cells were treated with various concentrations (6.25, 12.5, and 25 μg/mL) of curcumol for 24 h, the cells were harvested, washed twice with phosphate buffered saline (PBS), fixed in 4% paraformaldehyde at 4 °C for 24 h, and centrifuged. The pellet was treated with RNase (20 μg/mL) at room temperature for 30 min and then incubated with propidium iodide (PI, 50 μg/mL) for 30 min [10].

2.5. Detection of apoptosis

To determine the morphologic nuclear changes, cells were harvested and fixed with 4% paraformaldehyde 4 °C after the treatment with 50 μg/mL curcumol for 24 h. Jurkat cells were prepared and stained with Hoechst 33258 for 5 min at room temperature. Cells were washed

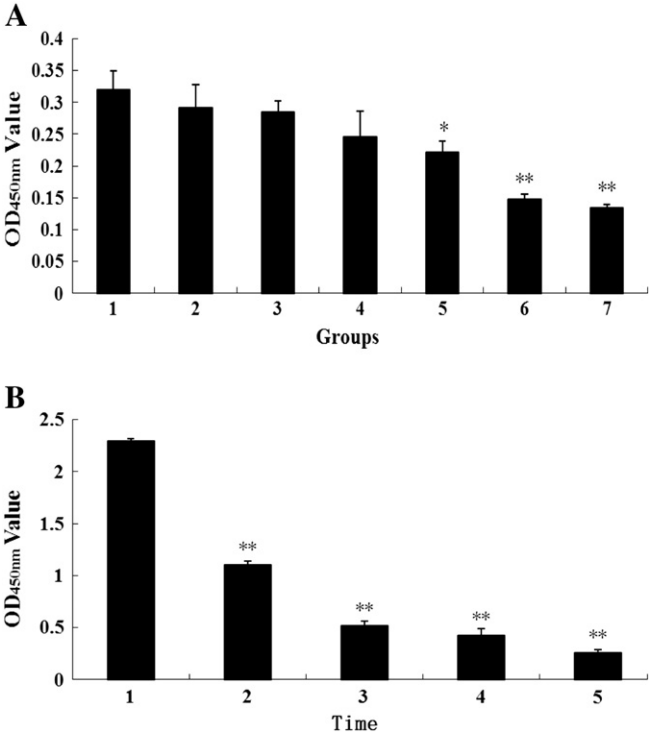

Fig. 1. A. Curcumol-induced concentration-dependent reduction of cell proliferation. 1, normal control; 2, IL-2 (50 ng/mL); 3, solvent control; 4, curcumol 6.25 μg/mL; 5, curcumol 12.5 μg/mL; 6, curcumol 25 μg/mL; 7, curcumol 50 μg/mL. Data are expressed as mean ± SD of triplicates. *$P < 0.05$, **$P < 0.01$, compared with IL-2. B. Curcumol-induced time-dependent reduction of cell proliferation. 1, normal control; 2, curcumol 50 μg/mL for 3 h; 3, curcumol 50 μg/mL for 6 h; 4, curcumol 50 μg/mL for 12 h; 5, curcumol 50 μg/mL for 24 h. *$P < 0.05$,**$P < 0.01$, compared with the normal control.