International Immunopharmacology 39 (2016) 79–83

Contents lists available at ScienceDirect

International Immunopharmacology

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

Therapeutic effect of dioscin on collagen-induced arthritis through reduction of Th1/Th2

Yachun Guo [a,1], Enhong Xing [b,1], Hongru Song [c,*], Guiying Feng [d], Xiujun Liang [e], Gao An [c], Xiaofei Zhao [c], Mi Wang [c]

[a] Department of Pathogen Biology, Chengde Medical College, Chengde 067000, Hebei, China
[b] Department of Central Lab, The Affiliated Hospital of Chengde Medical College, Chengde 067000, Hebei, China
[c] Department of Immunology, Chengde Medical College, Chengde 067000, Hebei, China
[d] Department of Clinical Nursing, Chengde Medical College, Chengde 067000, Hebei, China
[e] Department of Basic Medical Institute, Chengde Medical College, Chengde 067000, Hebei, China

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 24 June 2016
Accepted 24 June 2016
Available online 20 July 2016

Keywords:
Dioscin
Collagen-induced arthritis
Th1/Th2

ABSTRACT

The aim of this study was to detect the therapeutic effect of dioscin on collagen-induced arthritis (CIA). Mice model of CIA was induced by chicken collagen II and arthritis index was assessed. After suspension of dioscin (100 mg/kg/d) or triptolide was intragastrically administered, the left paw swelling and body weight of each mouse were measured. Then tissue samples were assayed by histopathological analysis. The levels of Th1 and Th2 were detected by flow cytometry. The expression of p-STAT1, p-STAT4 and p-STAT6 was demonstrated by western blot analysis, and T-bet and GATA-3 expression was detected by RT-PCR. The paw swelling and arthritis index were decreased and body weight was increased in the high dose of dioscin group compared to the model group ($P < 0.05$). Histopathological analysis revealed that the damage of synovium tissue in dioscin and triptolide group alleviated. The ratio of Th1/Th2 in the dioscin group (0.82 ± 0.24) and triptolide group (0.99 ± 0.44) was lower than that in the model group (1.84 ± 0.70, $P < 0.05$). Additionally, p-STAT4 expression was decreased, and both p-STAT6 and GATA3 expression was increased in the dioscin group than that in the model group ($P < 0.05$). Dioscin might have some therapeutic effects on CIA through regulating the proportion of Th1/Th2 cells, which could reduce the expression of p-STAT4, increase the expression of p-STAT6 and GATA3 in the synovial tissue.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that may bring about progressive joint degeneration, disability and is associated with cardiovascular complications which leads to mortality [1]. It is prevalent all over the world and seriously affects human physical and mental health [2]. The mechanisms of RA is associated with many factors, including infection, neuropsychological status, immune regulation, genetics and environmental factors [3]. In addition, persistent inflammatory process in RA is the result of disturbed B or T cell stimulation including autoreactive T cells [4].

Recently, various cytokine-targeted drugs have been used for the treatment of RA, including IL-1 receptor antagonists [5], nonsteroidal anti-inflammatory drugs [6] and tumor necrosis factor (TNF)-blockers (infliximab, etanercept, adalimumab, certolizumab pegol and golimumab) [7]. However, it still has undesirable side effects. For example, nonsteroidal anti-inflammatory drugs could cause peptic ulcers [8].

Dioscin is a natural product derived from *Dioscorea nipponica* Makino and *Dioscorea zingiberensis* Wright. Dioscin has been reported to have anti-inflammatory, anti-tumor, lipid-lowering, and hepatoprotective [9–11]. Additionally, dioscin exerts important roles in inhibiting osteoclast differentiation and bone resorption through down-regulating the Akt signaling cascades [12]. As another anti-inflammatory drugs, Triptolide can potently suppress the inflammatory responses and cartilage destruction in collagen-induced arthritis (CIA) mice [13]. Triptolide also could efficiently attenuate the severity of arthritis in CIA mice by reducing the mean arthritis index and the percentage of arthritic limbs [14]. However, to the best of our knowledge, the specific mechanism of dioscin on CIA has not been fully understood and whether triptolide has the same therapeutic effects as dioscin is largely unknown. Therefore, in the current study, we investigated the therapeutic effect of dioscin on CIA in mice, and studied the similar role of triptolide.

2. Materials and methods

2.1. Animal mode

A total of 120 male DBA1/J mice (7–8 weeks), weighting 18–22 g, were purchased from the Shanghai Silaike Laboratory Animal

* Corresponding author at: Department of immunology, Chengde Medical College, Anyuan road in Shuangqiao District of Chengde City, Hebei Province 067000, China.
E-mail address: songhhshhs@hotmail.com (H. Song).
[1] Co-first authors.

http://dx.doi.org/10.1016/j.intimp.2016.06.029
1567-5769/

Company (Shanghai, China). Animals were housed 5 per cage (600 × 450 × 280 mm) under an environment at 24 ± 1 °C, with relative humidity of 55 ± 5%, and a 12:12 h dark/light cycle under specific pathogen-free (SPF) conditions. All studies were performed in agreement with the Principles of Laboratory Animal Care and guidelines established by the Institutional Animal Care and Use Committee of the university.

After one week to adapt the environment, mice were randomly divided into 4 groups: control group (n = 20), model group (n = 20), dioscin at high dose group (100 mg/kg/d, n = 20), dioscin at middle dose group (50 mg/kg/d, n = 20), and dioscin at low dose group (25 mg/kg/d, n = 20), and triptolide group (n = 20). The chicken collagen II (2 mg/ml, Chondrex, Redmond, Washington, USA) was first emulsified with an equal volume of complete Freund's adjuvant (CFA, Difco; Sigma, USA) to a final concentration of 0.1 mg/ml collagen emulsion. Then, collagen emulsion (0.1 ml) was intradermally injected into the base of the tail of each mouse in the model, dioscin and triptolide group. On day 21, the same operation was performed. Meanwhile, mice in the control group were given an equal volume of normal saline.

2.2. Assessment of arthritis index

Paw volume of each mouse was measured every three days for signs of arthritis index between day 1 and 35 post-CFA. Arthritis was graded for severity of erythema and swelling using a 4-point scale (0 = normal, 1 = mild swelling and erythema of digits, 2 = swelling and erythema of the digits and toes joint, 3 = severe swelling and erythema of claws below the ankle, swelling and erythema of all the claws including the ankle) [15]. The total score of each mouse was calculated as the arthritic index, with a maximum possible score of 16 (4 points × 4 paws). The arthritis index ≥4 was considered as successful model.

2.3. Drug administration

The suspension of dioscin (purity > 98%, Chinese Nanjing Chunqiu Biological Engineering Co., Nanjing, China) or triptolide (Chinese Hubei Huangshifeiyun pharmaceutical company, Hubei, China) were first configured by 1% sodium carboxymethyl cellulose. Mice in the dioscin at high dose, middle dose and low dose groups was intragastrically administered with dioscin from the 7th day following the primary immunization at three levels, which are high (100 mg/kg/d), middle (50 mg/kg/d), and low dose (25 mg/kg/d), respectively, according to the dose of *Dioscorea nipponica* Makino (15 g/kg/d in human) and the content of dioscin in *Dioscorea nipponica* Makino (1.7–2%). The triptolide group was intragastrically administered triptolide suspension (17 μg/kg/d) according to the manufacturer's instructions. Control group and model group were intragastrically administrated with equal volume saline, respectively.

2.4. Evaluation of paw swelling and recording the body weight

During the course of treatment, the left paw volumes of each mouse were measured using a vernier caliper (GBT1214-1986, Shanghai, China). Each joint was measured for three times and averaged as the final record. The body weight was measured with a precision balance and the arthritis index was calculated according to the swelling of joint.

2.5. Samples collection and histopathological assessment

On day 35 during the drug administration, all the mice were sacrificed by cervical dislocation. Synovium tissue samples were collected from the joints of the mice immediately for histological, flow cytometry and immunohistochemistry test.

Mice synovium tissue was post-fixed overnight in 4% paraformaldehyde, embedded in paraffin, cut into 4 μm sections using a microtome and stained with hematoxylin and eosin (HE) to observe the inflammation infiltration and synovium destruction and estimate the inflammatory reaction in the synovium tissue.

2.6. Flow cytometry for Th1 and Th2 detection

Lymphocyte cell suspension (100 μl) from above mice in each group was incubated in the incubator, and collected into the tube. CD4-FITC (1 μl, eBioscience, San Diego, CA, USA) was added into each tube and incubated at room temperature for 20 min in the dark. The tube was added 100 μl buffer A, incubated at room temperature for 15 min in the dark, washed with PBS, and then added 100 μl hemolytic buffer B. Finally, anti-mouse IFN-γPE and anti-mouse IL-4 PE was added into the test 1 and test 2 tube, respectively. Cells were washed with PBS and measured by FACSCalibur flow cytometer (BD Biosciences Immunocytometry Systems, San Jose, CA, USA) according to the manufacturer's instructions. Flow cytometry was made in 19 replicates with 4 times independently.

2.7. Western blot analysis

The joint tissue of above mice was homogenized by adding 1:5 tissue weight of protein extraction buffer in a glass homogenizer. The protein concentration of joint tissue was determined using a BCA protein assay kit. Then, the proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) on 10% polyacrylamide gels and transferred to a polyvinylidene fluoride membrane. The membrane was blocked with 5% skim milk in Tris-buffered saline containing 0.05% Tween-20 (TBST) for 2 h, and special diluted primary rabbit anti- p-STAT1, p-STAT4 and p-STAT6 (1:1000, Abcam, Cambridge, UK) antibodies were added. After incubation overnight at 4 °C, the membrane was washed by TBST for three times, and Horseradish peroxidase labeled anti Ig antibody (Chinese Biyuntian Biotechnology Co., Jiangsu, China) was added and incubated for 1 h at room temperature. Finally, the samples were washed with TBST, and semi-quantitative gel image was analyzed by an UV gel imager (Shanghai Tianneng Co., Ltd., Shanghai, China). β-Actin was served as an internal reference.

2.8. RNA extraction and RT-PCR analysis

Total RNA was extracted from synovial tissue of above mice with TRIzol reagent (Ambion, Austin, TX, USA). Oligo(dT)-primed RNA was reverse transcribed to cDNA according to the manufacturer's instructions. The sequences of T-bet and GATA-3 used for amplification were as follows: T-bet forward, 5′-AGC AAG GAC GGC GAA TGT T-3′ and reverse, 5′-GGG TGG ACA TAT AAG CGG TTC-3′; GATA-3 forward, 5′-CTC GGC CAT TCG TAC ATG GAA-3′ and reverse, 5′-GGA TAC CTC TGC ACC GTA GC-3′; Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was served as an internal reference with forward 5′-AGG TCG GTG TGA ACG GAT TTG-3′ and reverse, 5′-TGT AGA CCA TGT AGT TGA GGT CA-3′. The conditions for PCR were set as follows: initial denaturation at 95 °C for 3 min, 30 s to denature at 95 °C, 72 °C for 45 s to extend and 40 cycles for amplification. The annealing temperatures were 57.5 °C. Finally, PCR products were analyzed by 1.5% agarose gel electrophoresis. Each sample was provided with 3 repeats.

2.9. Statistical analysis

All the results were expressed as mean ± standard deviation (SD) of the values obtained from four experiments and carried out by SPSS version 20.0 program. The comparisons between different groups were performed using one-way ANOVA. Statistically significant differences were defined as P values of <0.05.