Journal of Ethnopharmacology 146 (2013) 853–858

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Fuyuan Decoction inhibits nitric oxide production via inactivation of nuclear factor-κB in SW1353 chondrosarcoma cells

Ping Jia [a,*], Gang Chen [b], Guoqing Zhou [a], Yu Zhong [a], Rongheng Li [a]

[a] Department of Combination of Chinese and Western Medicine, the First Affiliated Hospital of Chongqing University of Medical Sciences, Chongqing 400016, PR China
[b] Research Center of Medical Chemistry and Chemical Biology, Chongqing Technology and Business University, Chongqing 400067, PR China

ARTICLE INFO

Article history:
Received 14 August 2012
Received in revised form
28 November 2012
Accepted 6 February 2013
Available online 1 March 2013

Keywords:
Fuyuan Decoction
Nitric oxide
Inducible nitric oxide synthase
Nuclear factor-κB
Traditional Chinese medicine

ABSTRACT

Ethnopharmacological relevance: Fuyuan Decoction (FYD) is an empirical formula of treating Bi Zheng in traditional Chinese medicine (TCM). Despite the fact that the efficiency of FYD on treating osteoarthritis has been verified in clinic, the underlying mechanisms are not totally understood. This study was to investigate the effects and mechanisms of FYD on nitric oxide (NO) production and nuclear factor (NF)-κB activation in interleukin (IL)-1β-stimulated chondrocytes.

Materials and methods: SW1353 human chondrosarcoma cells were pretreated with various concentrations of FYD-containing serum (FYD-CS), and then were stimulated by IL-1β. Amounts of NO were determined by Griess reaction assay. Inducible NO synthase (iNOS) expression, inhibitor-κBα (IκBα) degradation and nuclear translocation of p65 protein were determined by Western blot assay. DNA binding activity of NF-κB was determined by ELISA assay using Trans AM™ kit for p65.

Results: 10% and 20% (v/v) FYD-CS significantly decreased NO production in a concentration-dependent manner ($p < 0.05$ or $p < 0.01$) as compared to control in IL-1β-induced SW1353 cells. Besides, 10% and 20% FYD-CS also significantly reduced iNOS protein expression by about 60% and 70% (both $p < 0.01$), respectively. Furthermore, 10% and 20% FYD-CS markedly decreased IκBα degradation by about 45% and 26% ($p < 0.01$ or $p < 0.05$), lessened P65 content in the nucleus by about 28% and 60% (both $p < 0.01$), and repressed DNA binding activity of P65 by about 30% and 45% (both $p < 0.01$) in IL-1β-induced SW1353 cells.

Conclusions: These findings suggested that FYD could inhibit NO production and iNOS expression in IL-1β-induced chondrocytes through suppressing NF-κB activation.

1. Introduction

Osteoarthritis (OA), the rheumatic disease with the high prevalence and economic burden, is a degenerative disease involving chondrocytes, cartilage and other joint tissues. Studies in animal models and OA patients suggest that proinflammatory factors, including nitric oxide (NO), tumor necrosis factor (TNF)-α, Interleukin (IL)-1β, prostaglandin (PG) E_2, may be produced by the chondrocytes or by the synovium and other surrounding tissues, and that various pathways converge on the upregulation of aggrecanases, collagenases and matrix metalloproteinases (MMPs) resulting in joint destruction (Goldring and Otero, 2011).

There are three isoforms of nitric oxide synthase to catalyze the generation of NO, and in inflammatory condition, NO is principally synthesized by inducible nitric oxide synthase (iNOS) (Kobayashi, 2010). NO and iNOS are found upregulated in OA chondrocytes (Melchiorri et al., 1998), and high concentrations of nitrites and nitrates are detected in the synovial fluid (Karan et al., 2003) and plasma (Ersoy et al., 2002) of OA patients. NO inhibits both proteoglycan and collagen synthesis (Tomita et al., 2001), activates MMPs (Cake et al., 2003), induces chondrocyte apoptosis (Kim et al., 2002) and promotes inflammatory responses (Melchiorri et al., 1998). All of these functions contribute to the catabolic consequences of NO in cartilage.

A number of evidence demonstrates the contribution of abnormal nuclear factor-κB (NF-κB) activation on the onset and progress of OA disease (Marcu et al., 2010). NF-κB exists as a homo- or hetero-dimeric form of Rel family proteins and p65/p50

Abbreviations: Con-S, Control serum; COX-2, Cyclooxygenase-2; DMEM, Dulbecco's modified Eagle's medium; DMSO, Dimethyl sulfoxide; ECL, Enhanced chemiluminescence; FBS, Fetal bovine serum; FYD, Fuyuan Decoction; FYD-CS, FYD-containing serum; HPLC, High performance liquid chromatography; IκBα, Inhibitor-κBα; IKK, IκB kinase; IL-1β, Interleukin-1β; iNOS, Inducible nitric oxide synthase; MMPs, Matrix metalloproteinases; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF-κB, Nuclear factor-κB; NO, Nitric oxide; OA, Osteoarthritis; PG, Prostaglandin; PVDF, Polyvinylidene difluoride; SDS–PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; TCM, Traditional Chinese medicine; TNF-α, Tumor necrosis factor-α; v, Volume

* Correspondence to: Department of Combination of Chinese and Western Medicine, the First Affiliated Hospital of Chongqing University of Medical Sciences. No. 1, Yixueyuan Road, Yuzhong District, Chongqing 400016, PR China. Tel./fax: +86 23 8901 2864.
E-mail address: jiap008@yahoo.com.cn (P. Jia).

0378-8741/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2013.02.014

is the predominant heterodimer. NF-κB is sequestered in the cytoplasm, bound by inhibitor-κB (IκB) proteins, and activated in the nucleus after degradation of IκB by IκB kinase (IKK). Activated NF-κB regulates the expression of cytokines, chemokines, adhesion molecules, inflammatory mediators and matrix degrading enzymes, such as iNOS, cyclooxygenase-2 (COX-2), IL-1β, TNF-α and MMPs (Saklatvala, 2007). Concurrently, IL-1β and TNF-α also function as potent inducers for NF-κB activation.

In the context of traditional Chinese medicine (TCM), OA belongs to the category Bi Zheng which is defined as a syndrome marked by arthralgia and dyskinesia of the joints and limbs due to attack of the meridians of the limbs by wind, dampness, and heat or cold pathogens. Fuyuan Decoction (FYD) is an empirical formula of treating Bi Zheng in our clinical practice. Although FYD has been proved effective in OA treatment, the underlying mechanisms are not clear. The purpose of this study is to investigate the effects and mechanisms of FYD on NO production, iNOS expression and NF-κB activation in IL-1β-stimulated chondrocytes.

2. Materials and methods

2.1. Reagents

Naringin, icariin and astragaloside IV were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Recombinant human IL-1β was purchased from R&D System (Minneapolis, Minnesota, USA). Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (St Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Antibodies against iNOS, IκBα, NF-κB p65, β-actin and lamin B1 were purchased from Santa Cruz (Santa Cruz, CA, USA). Enhanced chemiluminescence (ECL) Western blot detection system and polyvinylidene difluoride (PVDF) membranes were purchased from Millipore (Bedford, MA, USA). Trans AM™ kit for p65 was purchased from Active Motif (Carlsbad, CA, USA). NO detection kit based on the Griess reaction was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

2.2. Preparation of FYD

15 g of *Epimedium brevicornu* (Berberidaceae), 15 g of *Astragalus membranaceus* (Leguminosae), 15 g of *Davallia formosana* (Davalliaceae), 15 g of *Psoralea corylifolia* (Leguminosae), 10 g of *Panax ginseng* (Araliaceae), 5 g of *Panax pseudo-ginseng var. notoginseng* (Araliaceae), 10 g of *Angelica sinensis* (Umbelliferae), 10 g of *Salvia miltiorrhiza* (Labiatae) and 5 g of *Glycyrrhiza uralensis* (Leguminosae) were purchased from Chongqing Tongjunge Pharmacy (Chongqing, China) and were identified by Dr. Jifen Zhang, college of pharmaceutical sciences, Southwest University (Chongqing, China). All the herbs were authenticated using thin-layer chromatography with reference to methods recommended by Chinese Pharmacopeia Commission (2010). These herb materials were extracted twice with boiling water (1:10, w/v) for 2 h, respectively. The solution was filtered, concentrated and then made into freeze-dried powder. The extraction yield of FYD was 20.3%. In this study, FYD was found to contain 1.9 mg naringin, 1.3 mg icariin and 0.09 mg astragaloside IV per g freeze-dried powders by high performance liquid chromatography (HPLC) method (Fig. 1).

2.3. Preparation of FYD-containing serum (FYD-CS)

Male Wistar rats weighing 220∼250 g were purchased from Chongqing University of Medical Sciences (Chongqing, China). All animal procedures were approved by the Ethical Committee in Animal Research of Chongqing University of Medical Sciences (CUMS11-66). Rats were randomly distributed into two groups: (1) FYD-CS group: 15 rats were orally administered 11 g/kg/d FYD twice daily (2 mL/rat) for 7 consecutive days. This dose based on the human equivalent dose in our clinical practice. (2) Control serum (Con-S) group: 15 rats were orally administered saline (2 mL/rat) twice daily for 7 consecutive days. Blood was taken from the abdominal artery 1 h after the last administration and then serum was separated by centrifugation of 2000 rpm for 20 min. Serum of the two groups was inactivated at 56° C for 30 min, filtered through a 0.22 μm filter and stored at −70 °C until use.

2.4. Cell culture

SW1353 human chondrosarcoma cell line purchased from the American Type Culture Collection. Cells were cultured in DMEM with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in humidified air with 5% CO_2.

2.5. MTT assay

SW1353 cells were incubated in 96-well plates with FYD-CS (2.5–40%) or Con-S (2.5–40%). After an incubation period of 48 h, MTT was added for 4 h at the final concentration of 0.5 mg/mL. Subsequently, the culture medium was removed and after

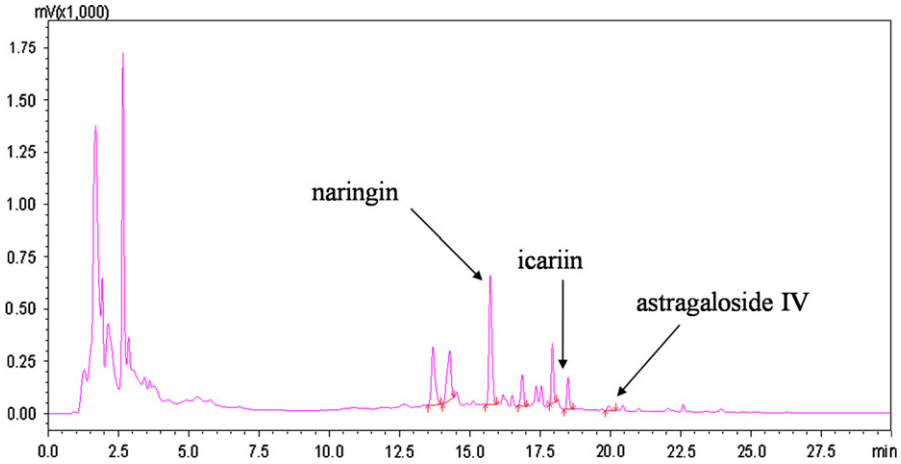

Fig. 1. HPLC Chromatograms of naringin, cariin and astragaloside IV in FYD.