Available online at www.sciencedirect.com
ScienceDirect
Life Sciences 81 (2007) 1677 – 1684
Life Sciences
www.elsevier.com/locate/lifescie

7′-(3′,4′-dihydroxyphenyl)-*N*-[(4-methoxyphenyl)ethyl]propenamide (Z23), an effective compound from the Chinese herb medicine *Fissistigma oldhamii* (Hemsl.) Merr, suppresses T cell-mediated immunity in vitro and in vivo

Xu-Dong Hu [a,1], Xiang-Gen Zhong [b,1], Xiang-Hua Zhang [c,1], Yi-Nan Zhang [c], Zong-Ping Zheng [c], Yu Zhou [b], Wei Tang [b], Yang Yang [a], Yi-Fu Yang [b], Li-Hong Hu [c,*], Jian-Ping Zuo [a,b,*]

[a] *Laboratory of Immunology and Virology, Shanghai University of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China*
[b] *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China*
[c] *Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China*

Received 1 March 2007; accepted 1 October 2007

Abstract

Fissistigma oldhamii (Hemsl.) Merr [*F. oldhamii*], a traditional Chinese herb medicine, is widely used for treating rheumatoid arthritis (RA) in China. Following bioactivity-guided isolation, a representative immunosuppressive compound with low cytotoxicity, 7′-(3′,4′-dihydroxyphenyl)-*N*-[(4-methoxyphenyl)ethyl]propenamide (Z23), was been identified in this herb medicine. We investigated the immunosuppressive effects of Z23 on T cells in vitro and in vivo. The results showed that Z23 in a dose-dependent manner significantly inhibited the proliferation of splenocytes induced by concanavalin A (ConA) and by the mixed lymphocyte culture reaction (MLR), with half inhibitive concentration (IC_{50}) values of 6.22 μM and 0.78 μM, respectively. Z23 also dose-dependently inhibited the proliferation and type 1 cytokine (IFN-γ and IL-2) production of primary T cells stimulated by anti-CD3/CD28 mAbs, but did not affect IL-12 production by mouse peritoneal macrophages (pMφ) stimulated with LPS plus IFN-γ in vitro. Administration of Z23 (6.25 mg/kg, 12.5 mg/kg, 25 mg/kg, i.p.) dose-dependently suppressed 2,4-dinitrofluorobenzene (DNFB)-induced delayed-type hypersensitivity (DTH) reactions. Furthermore, administration of Z23 (25 mg/kg, i.p.) significantly reduced the incidence and severity of type II bovine collagen (CII)-induced arthritis (CIA), which was associated with the inhibition of CII-specific T cell proliferation and type 1 cytokine (IFN-γ and IL-2) production. In this study, we report that a representative immunosuppressive compound from *F. oldhamii*, Z23, effectively inhibits murine immune responses in vitro and in vivo, and that the immunosuppressive effects of Z23 might be attributed to suppression of T cell activation and function and Th1 type cytokine production.

Keywords: Z23; Splenocyte proliferation; Mixed lymphocyte culture reaction; Delayed-type hypersensitivity; Collagen-induced arthritis

Introduction

Fissistigma oldhamii is a small or middle-sized vine, and its stem and root have been widely used in the treatment of autoimmune diseases in Traditional Chinese medicine, especially for rheumatoid arthritis (RA). However, its anti-RA mechanism is poorly understood.

In the etiological factors of RA, immune factors play an important role (Straub et al., 2002). The actions of Th-cells are of particular importance in RA. Th-cells are the dominant cell type in

* Corresponding authors. Zuo is to be contacted at Laboratory of Immunopharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, People's Republic of China. Tel./fax: +86 21 50806701. Hu, Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 199 Guo Shou Jing Road, Shanghai 201203, People's Republic of China. Tel./fax: +86 21 50802221.
E-mail addresses: simmhulh@mail.shcnc.ac.cn (L.-H. Hu), jpzuo@mail.shcnc.ac.cn (J.-P. Zuo).
[1] The authors contributed equally to this work.

0024-3205/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2007.10.004

the synovial fluid of patients with RA (Berner et al., 2000). Therefore, we speculated that the anti-RA effect of *F. oldhamii* may be related to its immunosuppressive effect on T cell-mediated immune responses.

To identify the most representative compound that might be responsible for the anti-RA effect of *F. oldhamii*, a series of alkaloids such as aporphines, oxoaporphines, and aristolactams from this herb were tested by bioactivity-guided isolation screening assays in vitro in our early work (Zhang et al., 2007). Some compounds showed good immunosuppressive effects, but their contents were very low in this herb. Through further research, we isolated a new compound, which was identified as 7′-(3′,4′-dihydroxyphenyl)-*N*-[(4-methoxyphenyl)ethyl]propenamide (Z23) (Fig. 1). The Z23 content is the highest among all of the isolated compounds from this herb and it is also easy to synthesize. The most important observation based on a ConA-induced splenocyte proliferation assay is that Z23 showed a very powerful immunosuppressive activity and low cytotoxicity. Therefore, we chose Z23 as the representative compound of *F. oldhamii* for further studies.

Following this line of research, we found that Z23 inhibited the murine T cell-mediated immune response effectively in vitro and in vivo. Since activated T lymphocytes play a key role in the onset and pathogenesis of several autoimmune diseases (Perkins, 1998), we tested the inhibitory activities of Z23 in T lymphocyte activation in vitro and in vivo. We observed its effects on mitogen- and alloantigen-induced splenocyte proliferation, anti-CD3/CD28 mAb stimulated-primary T cell proliferation and cytokine production in vitro, and its effects on T cell-mediated DNFB-induced DTH reaction and type II bovine collagen (CII)-induced arthritis (CIA) in vivo. We demonstrated that the immunosuppressive effects of Z23 might be attributed to suppression of T cell activation and function.

Materials and methods

Compounds

Z23 was extracted and purified from *F. oldhamii* (stem and root). The chemical structure of 7′-(3′,4′-dihydroxyphenyl)-*N*-[(4-methoxyphenyl)ethyl]propenamide (Z23) is shown in Fig. 1. The purity of Z23 was over 98% by HPLC analysis.

Reagents

RPMI (Roswell Park Memorial Institute) 1640 medium was purchased from GibcoBRL Life Technologies (USA). FBS (fetal bovine serum) was purchased from HyClone Laboratories (Logan, UT, USA). [^{3}H]-thymidine (1 mCi/ml) was purchased from the Shanghai Institute of Atomic Energy. Dimethyl sulfoxide (DMSO), concanavalin A (ConA), 3-[4,5-dimethylthylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Complete Freund's adjuvant (CFA) was purchased from Difco Laboratory (Detroit, MI, USA). 2,4-Dinitrofluorobenzene (DNFB) was purchased from PharMingen Co. (San Diego, CA, USA). CII was obtained from the Collagen Research Center (Tokyo, Japan), and all cytokine ELISA kits were obtained from BD PharMingen (San Diego, CA, USA).

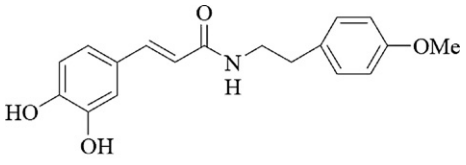

Fig. 1. The chemical structure of 7′-(3′,4′-dihydroxyphenyl)-*N*-[(4-methoxyphenyl) ethyl]propenamide (Z23).

Animals

Female BALB/c, C57BL/6 mice (6 to 8 weeks old) and DBA/1 (7 to 8 weeks old) mice were purchased from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences. The animals were housed in specific pathogen-free conditions (12 h light/12 h dark photoperiod, 22±1 °C, 55±5% relative humidity). All mice were allowed to acclimatize in our facility for 1 week before any experiments were started. All experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals, and were approved by the Bioethics Committee of the Shanghai Institute of Materia Medica.

Cell cultures

Mice were sacrificed, and their spleens were removed aseptically. A single-cell suspension was prepared, and cell debris and clumps were removed. Erythrocytes were lysed with Tris–NH_4Cl (0.155 M NH_4Cl and 16.5 mM Tris, pH 7.2) (Feng et al., 2002). Mononuclear cells were washed and resuspended in RPMI 1640 media supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.5 μM 2-mercaptoethanol.

MTT assay

Cytotoxicity was assessed by the MTT assay as previously described (Feng et al., 2004). Briefly, splenocytes were cultured in triplicate for 48 h with Z23. The cells cultured with medium alone were used as controls. MTT (5 mg/ml) reagent was added 4 h before the end of culture, and then cells were lysed with 10% sodium dodecyl sulfate (SDS) and 50% *N*,*N*-dimethylformamide, pH 7.2. O.D. values were read at 570 nm, and the percentage of viable cells was calculated.

ConA-induced proliferation assay

Splenic lymphocytes (4×10^5 cells/well) were cultured with 5 μg/ml of ConA plus Z23 in 96-well plates in triplicate for 48 h. Cells were pulsed with 0.5 μCi/well of [^{3}H]-thymidine for 8 h and harvested onto glass fiber filters. The incorporated radioactivity was then counted using a Beta Scintillation