Journal of Medicinal Plants Research Vol. 5(24), pp. 5789-5795, 30 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Triptolide inhibits cytokine-induced activation of retro-ocular fibroblasts from patients with Graves' ophthalmopathy

YAN Shu-xun[1], WANG Ying[2]*, Zhang bin[3], Feng Zhi-hai[4], Lv Jiu-sheng[4], Sun Ya-ru[4] and Lin Yan-jie[4]

[1]Department of Endocrinology and Metabolism, The First Affiliated Hospital, Henan College of Traditional Chinese Medicine, Zhengzhou City 450008, Henan province, People's Republic of China.
[2]Department of Geriatrics, The First Affiliated Hospital, Zhengzhou University, Zhengzhou City 450000, Henan province, People's Republic of China.
[3]Department of Chinese Medicine Pharmacology, Henan College of Traditional Chinese Medicine, Zhengzhou City 450008, Henan Province, People's Republic of China.
[4]Department of Endocrinology and Metabolism, The First Affiliated Hospital, Henan College of Traditional Chinese Medicine, Zhengzhou City 450008, Henan province, People's Republic of China.

Accepted 15 September, 2011

To explore the effects of Triptolide which has immunosuppressive and anti-inflammatory properties on the expression of human leucocyte antigen-DR (HLA-DR), intercellular adhesion molecule-1 (ICAM-1), CD40 and Fas enduced by cytokines and the synthesis of hyaluronic acid (HA) on cultured retro-ocular fibroblasts (RFs) from patients with Graves' ophthalmopathy. Cultured RFs were incubated with IL-1 (100 U/mL) or TNF-α (100 U/mL) in the presence of Triptolide. Flow cytometry was used to investigate the amount of HLA-DR, ICAM-1, CD40 and Fas. HA synthesis was measured by radioimmunoassay. At base conditions, the percentage of positive cells of HLA-DR, ICAM-1 and CD40 on RFs were 6.70 ± 3.06%, 14.89 ± 11.67% and 5.29 ± 3.02%, and the synthesis of HA was 337.8 ± 42.7 ng/ml. IL-1 or TNF-α significantly enhanced the amount of HLA-DR, ICAM-1 and CD40 and HA synthesis. Triptolide 0.01 µg/L had little effect on cytokine- induced HLA-DR, ICAM-1 and CD40 amounts, as well as HA synthesis. When the concentration ranged from 0.1 to 10 µg/L, Triptolide inhibited cytokine- induced RFs activation in a dose-dependent manner. Triptolide itself also induced Fas expression on RFs in a dose-dependent manner. Triptolide could inhibit cytokines-induced activation of RFs derived from patients with Graves' ophthalmopathy.

Key words: Triptolide, graves'ophthalmopathy, retro-ocular, fibroblasts, cytokine.

INTRODUCTION

Graves' ophthalmopathy (GO) is generally considered to be an organ-specific autoimmune disease. In this disease process, tissues become infiltrated with activated T lymphocytes and mast cells, and accumulate excessive amounts of glycosaminoglycan (GAGs, including hyaluronic acid [HA]) and the volume of the orbital fat compartments is increased (Daroszewski et al., 2006). This accumulation of inflammatory cells and GAGs in extraocular muscles and orbital connective tissue leads to the clinical manifestations of proptosis, diplopia and periorbital swelling (Modi et al., 2009). T lymphocytes which infiltrate retro-ocular tissue act upon retro-ocular fibroblasts (RFs) to stimulate cells proliferation, GAG synthesis and the expression of immunomodulatory molecules by releasing cytokines. Marked lymphocytic infiltration and inflammatory cytokines, such as interferon-γ (IFN-γ), interleukin-1 (IL)-1 and tumour necrosis factor-α (TNF-α), have been detected in active-stage orbital lesions (Gianoukakis et al., 2008). Aberrant expression of human leucocyte antigen-DR (HLA-DR) on fibroblasts has been implicated in the development of GO (Ponto et al., 2009; Yarman et al.,

*Corresponding author. E-mail: ysx982001@ yahoo.com.cn.

2007). In addition, the expression of various adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), on RFs is reportedly involved in the migration of lymphocytes to inflammatory sites in the orbit (Eckstein et al., 2009). CD40 is a critical signalling molecule expressed by B lymphocytes. It has been identified that CD40 expressed on RFs and plays an important role in the interaction between RFs and T lymphocytes (Hwang et al., 2009). The expression of HLA-DR, ICAM-1 and CD40 can be induced by the inflammatory cytokines. These results suggest that activation of RFss by proinflammatory cytokines may play an important role in the development of GO.

Triptolide, a diterpenoid triepoxide compound purified from the root of Tripterygium Wilfordii Hook F (TWHF) has been identified as one of the major components responsible for the immunosuppression qualities of the herb (Shui et al., 2010). We have previously reported that, triptolide suppressed the expression of HLA-DR, ICAM-1 and CD40 induced by IFN-γ on RFs derived from the patients with GO (Yan and Wang, 2006).

In the present study, we investigated the effects of triptolide on the expression of HLA-DR, ICAM-1, and CD40 induced by IL-1 and TNF-α on cultured human orbital fibroblasts derived from patients with GO, as well as the synthesis of HA. We also studied the effects of triptolide on Fas expression which mediates apoptosis of various cells (Apte et al., 2010).

MATERIALS AND METHODS

Drug

Source of Triptolide—triptolide (chemical structure, $C_{20}H_{24}O_6$; molecular weight, 360) was obtained from Fujian Medical Science Research Institute (Fujian, China). The material was composed of white to off-white crystals which had a melting point of 226 to 240°C, conformed to standard triptolide preparation by proton nuclear magnetic resonance and was found to be 99% pure by reverse phase high-performance liquid chromatography. A stock solution of triptolide was prepared by dissolving 5 mg of triptolide in 5 mL of dimethyl sulphoxide (DMSO) (Sigma, St. Louis, MO, USA) and then stored at −70°C. Final triptolide doses were diluted in a culture medium to a 1-mL volume containing treated doses.

Cell culture

Human retro-ocular connective tissues were obtained from two female patients undergoing orbital decompression surgery for severe active GO. One patient was 26 years old and had suffered from GO for 13 months; the second was 30 years old and had a 10 months history of GO.

The GO severity of two patients was grade IV according to the modified NOSPECS (N, no signs or symptoms; O, only signs, no symptoms; S, soft tissue involvement; P, proptosis; E, extraocular muscle involvement; C, corneal involvement; S, sight loss due to optic nerve involvement) score. Both patients had previously received glucocorticosteroids (not within 3 months before surgery), but had failed to respond or had experienced intolerable side-effects, were euthyroid and had not received radiotherapy to the orbits or other immunosuppressive treatment. Normal orbital connective tissue was derived from three individuals undergoing orbital surgery for strabism with no known history of GO or Graves' disease. Dermal fibroblasts were harvested by punch biopsy of skin from two normal individuals.

After explanation of the study, the authors obtained informed consent from all donors. Fibroblasts were cultured as previously reported. Briefly, all tissues were transported to the laboratory soon after removal. Then, the tissue was minced and placed in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Gaithersburg, MD, USA), supplemented with penicillin G (100 µ/mL) and streptomycin (0.1 mg/mL), and 10% foetal bovine serum (Life Technologies, Gaithersburg, MD, USA) on a tissue culture dish for the primary culture. The medium was changed routinely every 3 to 4 days. When fibroblasts were outgrown, the explants were removed. The fibroblasts monolayer was disrupted with 0.1% trypsin (Sigma) and 0.02% ethylenediaminetetraacetic acid (EDTA; Sigma), and the cells were re-plated. The cells were passaged every 10 to 15 days and were cultured further within a medium at 37°C in a humidified atmosphere of 5% CO_2. All cell strains used in the testing were taken from between the second and fifth passages, whose morphology and function had not changed. It was determined that these cells were morphologically consistent with a fibroblast phenotype and the expressions vimentin and collagen, two markers of fibroblast. At the same time, these cells failed to express factor VIII or smooth muscle-specific actin and thus the cultures were not contaminated with endothelial or smooth muscle cells (Yan and Wang, 2006).

Flow cytometer assay

Retro-ocular fibroblasts were seeded at 5×10^4 cells in each well of a 24-well plate (Coster). After 72 h, monolayer cells were switched to DMEM with 0.1% BSA for 24 h to reduce the effect of serum on the cells. The cells were further incubated in the medium alone or in IL-1 (100 U/mL) or TNF-α (100 U/mL) in the presence of various concentrations of triptolide for 48 h. Then, the cells were treated with 0.1% trypsin and 0.02% EDTA solution and incubated with mouse monoclonal antibody against HLA-DR (Immunotech, Marseilles, France), ICAM-1 (Immunotech), CD40 (Immunotech) and Fas (Immunotech) at 1:50–100 dilutions or with negative control mouse monoclonal antibody (immunoglobulin G-1 [IgG_1], IgG_2: Dako, Glostrup, Denmark) at a 1:50 dilution for 30 min on ice.

After washing with phosphate-buffered solution (PBS, 0.01 mol/L, pH 7.4), the cells were incubated for 30 min on ice with fluorescein isothiocyanate-conjugated rabbit IgG F(ab') 2 directed against heavy and light chains of mouse IgG (Wako Pure Chemical Industries, Osaka, Japan) at a 1:50 dilution. After a final wash, the cells were analysed by flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA). The cells were counted for 150 s or up to a minimum of 10 000 cells. Percentages of positive cells were determined. Fibroblasts whose immunofluorescence intensity was greater than that of fibroblasts stained with negative control mouse monoclonal antibody were classified as positive.

Radioimmunoassay

Retro-ocular fibroblasts were seeded at 5×10^4 cells in each well of a 24-well plate. After 72 h, cells were washed with PBS and continued in culture with DMEM supplemented 0.1% BSA and vitamin C 50 mg/L (Sigma) for 24 h to reduce the synthesis of basal HA. RFs were incubated in the medium alone or with the presence of IL-1(100 U/mL) or TNF-α(100 U/mL) and various doses of triptolide for 48 h and then the supernatants were collected in Epperdorf tubes and stored at −20°C. HA was measured by a radioimmunoassay method. HA radioimmunoassay kit s were

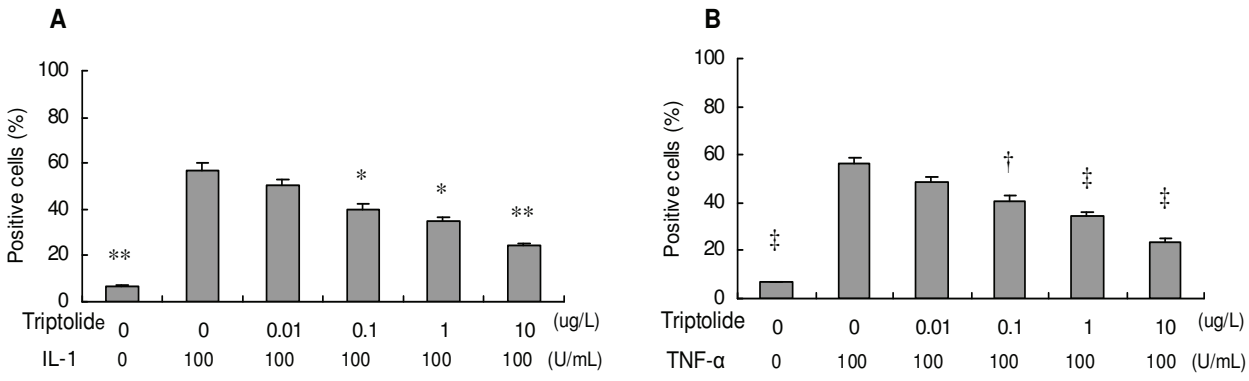

Figure 1. Effects of triptolide on the expression of human leucocyte antigen-DR induced by IL-1 (A), and TNF-α (B) on the surface of cultured retro-ocular fibroblasts from the patients with Graves' ophthalmopathy (n = 6; mean ± SD). Compare with IL-1, **P* < 0.05, ***P* <0.01; Compare with TNF-α, †*P* < 0.05, ‡*P* <0.01.

obtained from Shanghai Navy Medical Institute, Shanghai, China, and an SN-682B γ-radioimmunoassay counter was used to measure the content of HA.

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was determined by Student's *t*-test and *P*-value of 0.05 or less was considered statistically significant. All assays were performed on three cell lines in Triplicate. We found that there were no significantly different results between the cells obtained from different patients with GO. There were no significantly different results among the RFs from three normal individuals not suffering for GO and the skin fibroblasts from two normal subjects. This similarity was demonstrated by the reproducible results of repeated experiments. Thus, the data shown are the mean value for three repeats on three cell lines.

RESULTS

The effects of triptolide on the amount of HLA-DR induced by IL-1 or TNF-α on RFs from patients with GO

There was lower level of HLA-DR on RFs at base conditions. The value was 6.70 ± 3.06%. After incubation in IL-1 for 48 h, the quantity of HLA-DR increased significantly, and the value was 57.29 ± 15.68% (t = 7.758, P < 0.01, vs. control). Forty-eight-hour treatment with triptolide inhibited this effect of IL-1 in a dose-dependent fashion: triptolide 0.01 μg/L had little effect on the amount of HLA-DR 50.21 ± 14.97% (t = 0.800, P > 0.05, vs. IL-1); triptolide 0.1 μg/L suppressed it significantly, and the value was 40.16 ± 9.26% (t = 2.305, P < 0.05, vs. IL-1); triptolide 1 μg/L and 10 μg/L inhibited it further, and the values were 34.75 ± 10.37% (t = 2.938, P < 0.05, vs. IL-1) and 24.08 ± 9.19% (t = 4.477, P < 0.01, vs. IL-1), respectively (Figure 1A).

After incubation in TNF-α for 48 h, the quantity of HLA-DR increased significantly and the value was 56.17 ± 11.25% (t = 10.396, P < 0.01, vs. control). Forty-eight-hour treatment with triptolide inhibited this effect of TNF-α in a dose-dependent fashion: triptolide 0.01 μg/L had little effect on the amount of HLA-DR 48.34 ± 10.22% (t = 1.105, P > 0.05, vs. TNF-α); triptolide 0.1 μg/L suppressed it significantly, and the value was 40.58 ± 10.32% (t = 2.502, P < 0.05, vs. TNF-α); triptolide 1 and 10 μg/L inhibited it further, and the values were 34.12 ± 9.82% (t = 3.618, P < 0.01, vs. TNF-α) and 23.78 ± 8.39% (t = 5.655, P < 0.01, vs. TNF-α), respectively (Figure 1B).

The effects of triptolide on the amount of ICAM-1 induced by IL-1 or TNF-α on RFs from patients with GO

At basal condition, the level of ICAM-1 on RFs was 14.89 ± 11.67%. IL-1 enhanced it significantly, and the value was 79.41 ± 14.92% (t = 8.328, P < 0.01, vs. control). Triptolide 0.01 μg/L had little effect on IL-1 effect, and the values were 70.23 ± 12.41% (t = 1.157, P > 0.05, *vs.* IL-1). When the concentration increased from 0.1 to 10 μg/L, triptolide inhibited the induced effect of IL-1 in a dose-dependent manner, the value was 59.29 ± 10.11% (t = 2.729, P < 0.05, *vs.* IL-1), 46.17 ± 8.69% (1 μg/L, t = 4.701, P < 0.01, *vs.* IL-1) and 31.56 ± 7.76% (10 μg/L, t = 6.953, P < 0.01, *vs.* IL-1), respectively (Figure 2A).

After incubation in TNF-αfor 48 h, the quantity of ICAM-1 increased significantly and the value was 56.73 ± 13.91% (t = 5.646, P < 0.01, vs. control). Triptolide 0.01 μg/L had little effect on TNF-α effect, and the values were 50.11 ± 12.07% (t = 0.881, P > 0.05, *vs.* TNF-α). When the concentration increased from 0.1 to 10 μg/L, triptolide inhibited the induced effect of IL-1 in a dose-dependent manner, the value was 40.21 ± 10.23% (t = 2.344, P < 0.05, *vs.* TNF-α), 32.58 ± 8.25% (1 μg/L, t = 3.659, P < 0.01, *vs.* TNF-α) and 20.04 ± 7.34% (10 μg/L, t = 5.715, P < 0.01, *vs.* TNF-α), respectively (Figure 2B).

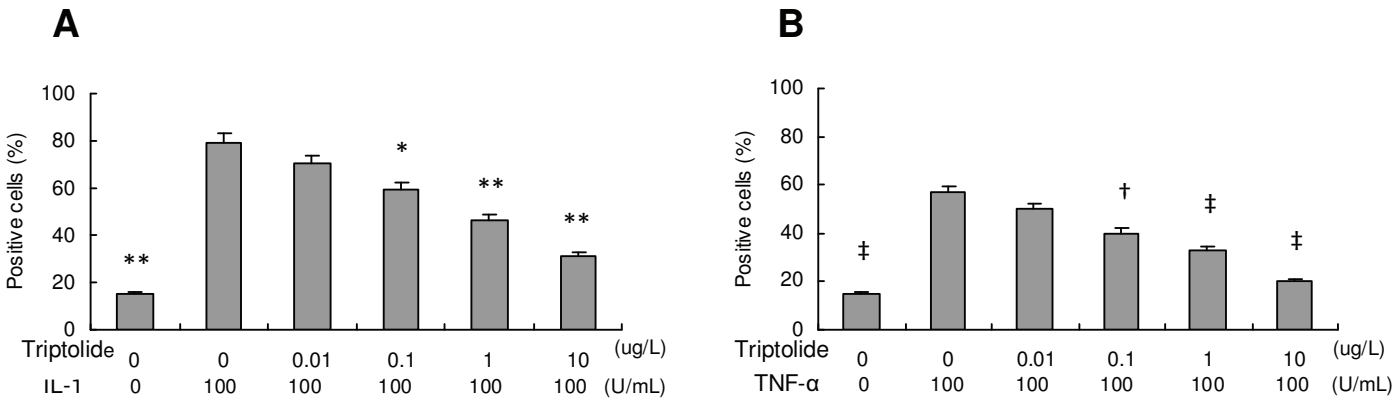

Figure 2. Effects of triptolide on the expression of intercellular adhesion molecule-1 induced by IL-1 (A), and TNF-α (B) on the surface of cultured retro-ocular fibroblasts from the patients with Graves' ophthalmopathy (n = 6; mean ± SD). Compare with IL-1, **P* < 0.05, ***P* <0.01; Compare with TNF-α, †*P* < 0.05, ‡*P* <0.01.

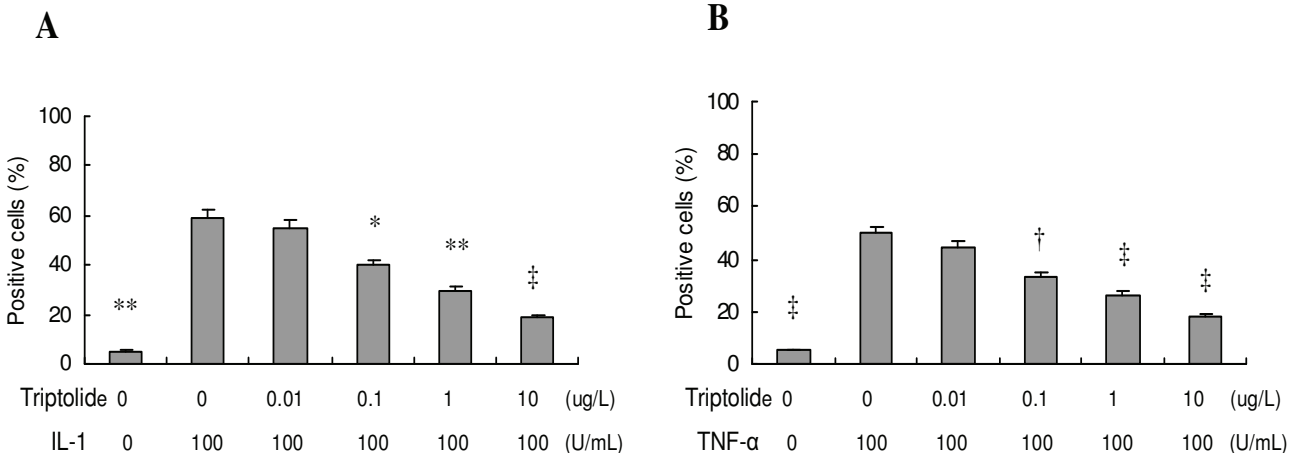

Figure 3. Effects of triptolide on the expression of CD40 induced by IL-1 (A), and TNF-α (B) on the surface of cultured retro-ocular fibroblasts from the patients with Graves' ophthalmopathy (n = 6; mean ± SD). Compare with IL-1, **P* < 0.05, ***P* <0.01; Compare with TNF-α, †*P* < 0.05, ‡*P* <0.01.

The effects of triptolide on the amount of CD40 induced by IL-1 or TNF-α on RFs from patients with GO

At basal condition, the level of CD40 was 5.29 ± 3.02% on RFs. Compared with base values, IL-1 increased the quantity to 59.17 ± 14.16% (t = 9.117, P < 0.01). Triptolide 0.01 μg/L had little effect on the amount of CD40 55.23 ± 12.36% (t = 0.514, P > 0.05, *vs.* IL-1). When the concentration increased from 0.1 to 10 μg/L, triptolide inhibited the induced effect of IL-1 in a dose-dependent manner and the values were 40.04 ± 8.92% (0.1 μg/L, t = 2.801, P < 0.05, *vs.* IL-1), 29.48 ± 9.01% (1 μg/L, t = 4.334, P < 0.01, *vs.* IL-1) and 18.77 ± 8.65% (10 μg/L, t = 5.966, P < 0.01, *vs.* IL-1), respectively (Figure 3A).

After incubation in TNF-αfor 48 h, the quantity of CD40 increased significantly and the value was 50.09 ± 12.14% (t = 8.774, P < 0.01, vs. control). Triptolide 0.01 μg/L had little effect on TNF-α effect, and the values were 44.61 ± 11.17% (t = 0.814, P > 0.05, *vs.* TNF-α). When the concentration of triptolide increased from 0.1 to 10 μg/L, triptolide inhibited the induced effect of IL-1 in a dose-dependent manner, the value was 33.45 ± 8.38% (t = 2.760, P < 0.05, *vs.* TNF-α), 26.33 ± 7.51% (1 μg/L, t = 4.078, P < 0.01, *vs.* TNF-α) and 18.05 ± 7.32% (10 μg/L, t = 5.537, P < 0.01, *vs.* TNF-α), respectively (Figure 3B).

The effects of triptolide on Fas expression on RFs from patients with GO

Although, cytonike induced Fas expression on fibroblasts, triptolide itself induced Fas expression on RFs in a dose-dependent manner (Figure 4).

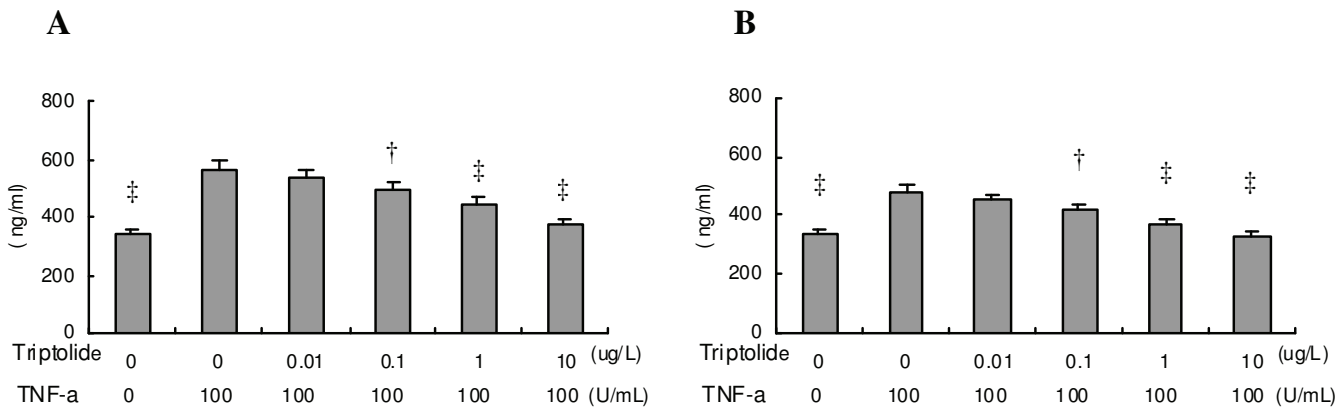

Figure 4. Effects of triptolide on hyaluronic acid synthesis of retro-ocular fibroblasts induced by IL-1 (A), and TNF-α (B) on the surface of cultured retro-ocular fibroblasts from the patients with Graves' ophthalmopathy (n = 6; mean ± SD). Compare with IL-1, **P* < 0.05, ***P* <0.01; Compare with TNF-α, †*P* < 0.05, ‡*P* <0.01.

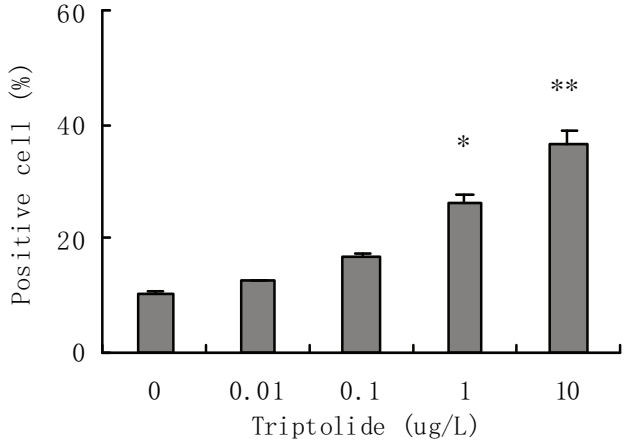

Figure 5. Effects of triptolide on Fas expression on the surface of cultured retro-ocular fibroblasts from the patients with Graves' ophthalmopathy (n = 6; mean ± SD). Compare with contro, **P* < 0.05, ***P* <0.01.

The effects of triptolide on the HA synthesis induced by IL-1 or TNF-α on RFs from patients with GO

At basal condition, the mount of HA was (337.8 ± 42.7) ng/ml. After incubation in IL-1 alone for 48 h, HA synthesis increased significantly, the value was (560.2 ± 38.4) ng/ml (*t* = 8.794, *P* < 0.01, *vs.* control). Triptolide 0.01 μg/L had little effect on IL-1 effect and the values were (534.6 ± 38.4) ng/ml (*t* = 1.062, *P* > 0.05, *vs.*IL-1). When the concentration of triptolide increased from 0.1 to 10 μg/L, triptolide inhibited the induced effect of IL-1 in a dose-dependent manner, the value was (489.1 ± 40.1) ng/ml (*t* = 2.894, *P* < 0.05, *vs.* IL-1), (441.2 ± 34.5) ng/ml (1 μg/L, *t* = 5.149, *P* < 0.01, *vs.* IL-1) and (373.6 ± 32.5) ng/ml (10 μg/L, *t* = 8.248, *P* < 0.01, *vs.* IL-1), respectively, (Figure 5A).

After incubation in TNF-αalone for 48 h, HA synthesis increased significantly, the value was (483.2 ± 43.4) ng/ml (*t* = 5.581, *P* < 0.01, *vs.* control). Triptolide 0.01 μg/L had little effect on TNF-αeffect, and the values were (450.4 ± 44.7) ng/ml (*t* = 1.290, *P* > 0.05, *vs.* TNF-α). When the concentration of triptolide increased from 0.1 to 10 μg/L, triptolide inhibited the induced effect of TNF-αin a dose-dependent manner, the value was (415.7 ± 40.2) ng/ml (*t* = 2.796, *P* < 0.05, *vs.* TNF-α), (370.1 ± 35.8) ng/ml (1 μg/L, *t* = 4.926, *P* < 0.01, *vs.* TNF-α) and (328.5 ± 30.3) ng/ml (10 μg/L, *t* = 7.161, *P* < 0.01, *vs.* TNF-α), respectively (Figure 5B).

The effects of triptolide on the RFs from normal individuals not suffering for GO

To investigate whether the effects of triptolide on the level of HLA-DR, ICAM-1, CD40, Fas and HA synthesis were specific to the patients with GO, we repeated the experiments using RFs from normal individuals. Compared with IL-1 group, triptolide inhibited IL-1-induced amount of HLA-DR, ICAM-1, CD40 and HA synthesis in a dose-dependent manner. When triptolide was 10 μg/L, the mounts of HLA-DR was 22.73 ± 7.25% (*vs* 52.91 ± 14.11%, *t* = 4.661, *P* < 0.01), ICAM-1 was 30.96 ± 9.18% (*vs* 74.23 ± 13.54%, *t* = 6.496, *P* < 0.01), CD40 was 21.07 ± 7.48% (*vs* 53.78 ± 13.69%, *t* = 5.137, *P* < 0.01), the mounts of HA was 362.3 ± 34.5 ng/ml (*vs* 526.7 ± 46.1 ng/ml, *t* = 7.024, *P* < 0.01). Compared with TNF-αgroup, triptolide inhibited TNF-α-induced amount of HLA-DR, ICAM-1, CD40 and HA synthesis in a dose-dependent manner. When triptolide was 10 μg/L, the mounts of HLA-DR was 20.45 ± 8.07% (*vs* 54.17 ± 14.18%, *t* = 4.988, *P* < 0.01), ICAM-1 was 20.08 ± 9.17% (*vs* 52.91 ± 12.84%, *t* = 5.908, *P* < 0.01) ,CD40 was 15.29 ± 7.49% (*vs* 46.29 ± 13.94%, *t* = 4.799, *P* < 0.01), the

mounts of HA was 360.6 ± 34.9 ng/ml (*vs* 489.4 ± 46.8 ng/ml, t = 5.405, P < 0.01). Additionally, triptolide enhanced Fas expression on orbital fibroblasts from normal individuals (basal, 11.23 ± 5.19%; triptolide 10 µg/L, 34.87 ± 13.27%, t = 4.065, P < 0.01).

The effects of triptolide on skin fibroblasts from normal subjects

To determine whether the effects of triptolide on the level of HLA-DR, ICAM-1, CD40, Fas and HA synthesis are specific to the RFs, we repeated the experiments using skin fibroblasts from normal subjects. Compared with IL-1 group, triptolide inhibited IL-1-induced amount of HLA-DR, ICAM-1, CD40 and HA synthesis in a dose-dependent manner. When triptolide was 10 µg/L, the mounts of HLA-DR was 23.48 ± 8.51% (*vs* 54.29 ± 14.17%, t = 4.567, P < 0.01),

ICAM-1 was 34.17 ± 8.36% (*vs* 75.94 ± 14.87%, t = 5.999, P < 0.01), CD40 was 22.45 ± 8.91% (*vs* 56.23 ± 14.28%, t = 4.917, P < 0.01), the mounts of HA was 389.3 ± 35.2 ng/ml (*vs* 544.9 ± 43.7 ng/ml, t = 6.794, P < 0.01). Compared with TNF-αgroup, triptolide inhibited TNF-α-induced amount of HLA-DR, ICAM-1, CD40 and HA synthesis in a dose-dependent manner. When triptolide was 10 µg/L, the mounts of HLA-DR was 24.72 ± 9.38% (*vs* 52.55 ± 12.39%, t = 5.265, P < 0.01), ICAM-1 was 22.38 ± 8.93% (*vs* 54.21 ± 13.69%, t = 4.771, P < 0.01), CD40 was 16.71 ± 8.62% (*vs* 48.92 ± 12.78%, t = 5.119, P < 0.01), the mounts of HA was 345.6 ± 33.8 ng/ml (*vs* 477.7 ± 41.2 ng/ml, t = 6.703, P < 0.01).On the other hand, triptolide enhanced the Fas expression on skin fibroblasts (basal, 10.44 ± 5.48%; triptolide 10 µg/L, 35.35 ± 14.13%, t = 4.027, P < 0.01).

DISCUSSION

Tripterygium Wilfordii Hook F (TWHF) has been reported to be effective in the treatment of a variety of autoimmune disorders including rheumatoid arthritis, systemic lupus erythematosus, nephritis and asthma (Sun et al., 2010; Matta, 2009). The major active component of this herb is triptolide and most of the efficacy of this herb's immunosuppression is attributed to triptolide (Shui et al., 2010). Previous works showed that triptolide could induce apoptosis of T cells, inhibit the expression of immunomodulatory molecules (Li et al., 2002). In the present study, it was observed that triptolide had an inhibitory effect on cytonike-induced HLA-DR, ICAM-1, CD40 and Fas expressions on RFs from the patients with GO and the same effects on the synthesis of HA.

There are indications that the expressions of HLA-DR and ICAM-1 on RFs may play important roles in the pathogenesis of GO ((Ponto et al., 2009; Eckstein et al., 2009). The expressions of HLA-DR and ICAM-1 and ICAM-1-mediated lymphocyte binding are induced by many cytokines such as IFN-γ, IL-1, TNF-α. Because ICAM-1 is involved in directing lymphocytes from the blood to inflammatory sites and also augments immune processes such as antigen presentation and cytotoxicity (Zhao et al., 2000), the suppression of ICAM-1 expression on RFs by triptolide may reduce the inflammatory reactions associated with GO.

CD40 as member of TNF-α receptor superfamily is expressed on RFs from the patients with GO and represents a pathway for interaction between these fibroblasts and CD40 ligand-expressing cells, such as T lymphocytes and mast cells. Engagement of CD40 with CD40 ligand leads to activation in T lymphocytes and RFs and an increase in HA synthesis, which can result in the tissue remodelling observed in GO. Thus, the CD40/CD40 ligand bridge represents a potentially important activation pathway for RFs that may underlie the cross-talk between these cells and leucocytes. Many cytokines could upregulate the expression of CD40 and increase the synthesis of HA significantly (Hwang et al., 2009; Zhao et al., 2010). In the present study, it was found that triptolide suppressed the expression of CD40 on RFs which was useful in preventing the activation in T lymphocytes and RFs decreasing the synthesis of HA. Fas-mediated apoptosis a type of cell death is involved in control of cell proliferation. Fas ligand is expressed in activated T cells and Fas expression is enhanced by various inflammatory cytokines (Apte et al., 2010). It were reported previously that the presence of Fas ligand and Fas expression in the orbital tissue from patients with TAO (Myśliwiec et al., 2004). In the present study, we demonstrated that the expression of Fas on RFs was enhanced by triptolide.

Triptolide have been used successfully in traditional Chinese medicine for more than two thousand years although the toxicities of triptolide may be associated with renal, cardiac, hematopoietic and reproductive systems (Chen, 2001). Currently, available data suggest that triptolide is safely for the treatment of a variety of autoimmune diseases and transplantation. In our previous studies, RFs incubation with triptolide (0.01, 0.1, 1, 10 µg/L) for 48 h was not detrimental to cell viability. Incubation with 20 µg/L triptolide decreased cell viability and greater toxicity was observed with 100 µg/L triptolide. Because of the results, all subsequent experiments were limited to 48-h treatments with the concentrations of triptolide (0.01, 0.1, 1, 10 µg/L).

In conclusion, triptolide inhibited the expressions of HLA-DR, ICAM-1, CD40 and Fas induced by cytokines such as IFN-γ, IL-1, TNF-α on RFs derived from the GO patients as well as the synthesis of HA, and had the same inhibiting effects on those cytokines-induced normal RFs and skin fibroblasts from patients with normal individuals. This shows that the effects of triptolide are not specific to RFs from patients with GO. Due to these suppressive effects, triptolide is potentially able to modify the antigen presentation and the HA synthesis by RFs.

ACKNOWLEDGEMENTS

This work was sponsored by Program for Science and Technology Innovation Talents in Henan College of Chinese Medicine and Program for Science and Technology Innovation Talents in Henan Health Department. We thank Dr Randy Green, Department of English, Zhengzhou University School of Medicine, China for critical reading of the manuscript.

REFERENCES

Apte A, Bonchev D, Fong S (2010). Cellular Automata Modeling of FASL-Initiated Apoptosis. Chem. Biodivers, 5: 1163-1172.

Chen BJ (2001). Triptolide, a novel immunosuppressive and anti-inflammatory agent purified from a Chinese herb Tripterygium wilfordii Hook F. Leuk. Lymphoma, 3: 253-265.

Daroszewski J, Rybka J, Gamian A (2006). Glycosaminoglycans in the pathogenesis and diagnostics of Graves's ophthalmopathy. Postepy Hig. Med. Dosw., 60: 370-373.

Eckstein AK, Johnson KT, Thanos M, Esser J, Ludgate M (2009). Current insights into the pathogenesis of Graves' orbitopathy. Horm. Metab. Res., 6: 456-464.

Gianoukakis AG, Khadavi N, Smith TJ (2008). Cytokines, Graves' disease, and thyroid-associated ophthalmopathy. Thyroid, 9: 953-958.

Hwang CJ, Afifiyan N, Sand D, Naik V, Said J, Pollock S, Chen BL, Phipps RP, Goldberg RA, Smith TJ, Douglas RS (2009). Orbital fibroblasts from patients with thyroid-associated ophthalmopathy overexpress CD40: CD154 hyperinduces IL-6, IL-8, and MCP-1. Invest. Ophthalmol. Vis. Sci., 5: 2262-2268.

Li H, Liu ZH, Dai CS, Liu D, Li LS (2002). Triptolide inhibits proinflammatory factor-induced over-expression of class II MHC and B7 molecules in renal tubular epithelial cells. Acta Pharmacol. Sin., 23: 775-781.

Modi NC, James J, Sleep T (2009).Thyroid eye disease. J. Perioper Pract., 9: 282-286.

Myśliwiec J, Kretowski A, Stepień A, Okłota M, Kinalska I (2004). Serum Fas in patients with Graves' ophthalmopathy as a marker of activity of the ocular inflammatory infiltration. Pol. Merkur Med., 100: 368-370.

Ponto KA, Pitz S, Mann WJ, Weber MM, Pfeiffer N, Kahaly GJ (2009). Management of Graves' orbitopathy: evidence-based recommendations. Dtsch. Med. Wochenschr., 49: 2521-2524.

Shui G, Wan Y, Jiang CI (2010). Progress in Tripterygium wilfordiiand its bioactive components in the field of pharmacodynamics and pharmacology. Zhongguo Zhong Yao Za Zhi, 4: 515-520.

Sun S, Wang Y, Zhou Y (2010). Research progress on immunosuppressive activity of monomers extracted from Chinese medicine. Zhongguo Zhong Yao Za Zhi, 3: 393-396.

Yan SX, Wang Y (2006). Inhibitory Effects of triptolide on IFN-γ Induced HLA-DR, ICAM-1, CD40 Expression on Retroocular Fibroblasts Derived from Patients with Graves'Ophthalmopathy. Clin. Exp. Ophthalmol., 3: 265-271.

Yarman S, Oguz F, Carin M (2007). HLA-DRB1*03 is a susceptibility gene in patients with Graves' disease with and without ophthalmopathy. Int. J. Immunogenet., 1: 23-25.

Zhao LQ, Wei RL, Cheng JW, Cai JP, Li Y (2010). CD40-CD40L ligand signaling induces intercellular adhesion molecule-1 expression in orbital fibroblasts in patients with Graves' ophthalmopathy. Invest. Ophthalmol. Vis. Sci., 51: 4652-4660.

Zhao Q, Liu Y, Li Q (2000). The effects of Triptolide on HLA antigens expression of corneal epithelial cells induced by interferon-*gamma in vitro*. Yan Ke Xue Bao, 16: 34-37.