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Original Research Article

Hederagenin inhibits proliferation, angiogenesis and inflammation of fibroblast-like synovial cells in rheumatoid arthritis

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Abstract

Purpose: *To determine the effect of Hederagenin (Hed) on rheumatoid arthritis (RA) in a cell model, and to elucidate the mechanism of action of Hed.*
Methods: *MTT, EDU, and Immunoblot assays were used to determine the effects of Hed on the viability of fibroblast-like synovial cells, while the effects of Hed on inflammation were examined by enzyme-linked immunosorbent assay (ELISA) and immunoblot assay. The influence of Hed on cell motility angiogenesis was evaluated by Transwell and tube formation assays, while immunoblot analysis was used to determine the mechanism of action of Hed.*
Results: *Hed inhibited the viability of RA-FLS cells and suppressed the inflammation of RA-FLS cells ($p < 0.05$). Furthermore, Hed suppressed the migration and angiogenesis of RA-FLSl cells, as well as regulated MAPK pathway ($p < 0.05$).*
Conclusion: *Hed inhibits the proliferation, angiogenesis and inflammation of fibroblast-like synovial cells in RA by regulating MAPK pathway. Therefore, Hed is a drug for the treatment of RA, However, in vivo studies to validate these findings are recommended.*

Keywords: *Rheumatoid arthritis, Hederagenin, Inflammation, Angiogenesis, MAPK pathway*

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INTRODUCTION

Rheumatoid arthritis (RA) is a type of chronic autoimmune disease characterized by joint inflammation, cartilage damage and joint destruction that negatively affects patients' physical movement and quality of life [1,2]. It has been suggested that the pathogenesis of RA is related to non-controlled autoimmunity as well as the resulting inflammation of antigen-presenting cells and lymphocytes [3]. Angiogenesis is the formation of new vasculature systems [3,4]; however, angiogenesis is vital in a variety of pathological conditions [5-7].

In RA, angiogenesis occurs at the earliest stages of the disease and is considered a transition to chronic inflammation [8,9]. Chronic inflammation maintains blood vessel growth through the secretion of angiogenic factors, and synovial angiogenesis may exacerbate inflammation [10].

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Hederagenin (Hed) is the main active ingredient of *Tripterygium wilfordii* [11]. Hed has many physiological functions. Hed plays a neuroprotective role in protecting PC12 cells from corticosterone-induced damage by activating PI3K/AKT pathway. Hed also lowers blood lipids, especially TG clearance [12]. This mechanism of action may be related to the inhibition of lipid absorption and down-regulation of p38 phosphorylation [13]. Hed also ameliorates cisplatin-induced acute kidney injury and inflammatory response by inhibiting Axin2/β-catenin pathway [14]; in addition, may be a potential anti-osteoarthritis drug. Hed significantly reduced IL-1β-induced apoptosis and ROS accumulation. Hed also had anti-apoptotic and antioxidant effects [15]. However, whether Hed has a therapeutic effect on RA, as well as the potential mechanism, is still not known. In this study, the probable effect of Hed on rheumatoid arthritis was investigated.

EXPERIMENTAL

Cell culture

Human RA-FLS cells were purchased from Chinese Academy of Sciences and maintained with the RPMI-1640 complete medium, and treated with IL-1β (Sigma) or Hed (Sigma) for 24 h at a concentration of 0, 1, 25 or 50 Um, or 1 μM Tofacitinib (Sigma). Edu kit (Abcam) was used to assess the effect of Hed.

Western blotting assay

RIPA lysate (Beyotime, Shanghai, China) was used to lysate the cells to extract protein. The total proteins were quantified using BCA reagent, separated by SDS-PAGE, and then transferred onto PVDF membranes. The proteins were blocked with 5 % milk for 1 h.

Primary antibodies, including anti-p-ERK antibody (1:1000, ab201015), anti-ERK antibody (1:1000, ab184699), anti-p-JNK antibody (1:500, ab215208), anti-JNK antibody (1:500, ab110724) anti-p-p38 antibody (1:1000, ab17886) anti-p38 antibody (1:1000, ab170099), β-actin (1:3000; ab8226) at room temperature for 2 h, and then secondary antibodies were incubated for 1 h and photographed after chemiluminescence. Relative protein level was measured by ImageJ (USA) and normalized to the expression of β-actin.

CCK-8 assay

RA-FLS cells (1000 cells/well) were plated into 96-well plates, maintained for 48 h, and subsequently incubated with CCK-8 (Beyotime, China) for 4 h. Then, the absorbance was spectrophotometrically measured at 450 nm using a microplate reader (BD, USA).

Edu assay

The Edu-treated RA-FLS cells were fixed and blocked in 10 % goat serum for 1 h. Then, the cells were stained with DAPI for 3 min. Finally, after washing in PBS, sections were examined by a microscope (Leica 5000B; Germany).

Transwell assay

The RA-FLS cells were seeded into the upper chambers of Transwell facility (BD, USA) and allowed to migrate (with or without the 20 % matrigel) for 24 h. The invaded cells on the upper chamber were fixed, stained with 2 % crystal violet, and the images were captured. The effect on cell invasion was determined by counting stained cells.

Enzyme-linked immunosorbent assay (ELISA)

CXCL10, IL-8 and IL-6 were determined by ELISA (Abcam, UK). Standard reagents and samples were mixed and incubated for 20 h at 4 °C. TMB solution was added to the cells. Absorbance was measured spectrophotometrically at 450 nm in a microplate reader (Bio-Rad, Hercules, CA).

Statistical analysis

GraphPad 5.0 software was used for statistical analysis, and the data presented as mean ± SD, and analyzed by t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Hed inhibited the proliferation of RA fibroblast-like synovial cells

IL-1β was first used to simulate RA in RA-FLS *in vitro*. IL-1β enhanced the viability of RA-FLS cells (Figure 1 A). Hed treatment inhibited the viability in RA-FLS cells when treated with IL-1β, similar to treatments with tofacitinib and positive control (Figure 1 A).

The results from Edu assays showed Hed treatment decreased the Edu-positive cell numbers in RA-FLS cells upon the treatment with IL-1β, which was similar to treatment with tofacitinib (Figure 1 B and C). Thus, Hed inhibited the growth of RA-FLS cells

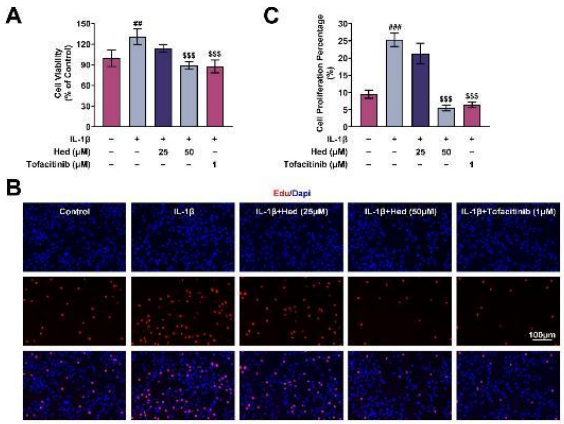

Figure 1: Hed inhibited the proliferation of RA fibroblast-like synovial cells. (A) Effect of Hed, with or without IL-1β, on RA-FLS cells. 1 μM Tofacitinib treatment was the positive control. (B) Edu assays showed effect of Hed on the viability of RA-FLS cells (C). Edu-positive cell quantification of panel B. Data are presented as mean ± SD; $^{\#\#}p$<0.01, $^{\#\#\#}p$ < 0.001, IL-1β vs control; $^{\&\&\&}p$ < 0.001, IL-1β+Hed vs IL-1β

Hed suppressed RA fibroblast-like synovial cells

Figure 2 shows the effect of Hed on the inflammation of RA fibroblast-like synovial cells upon treatment with IL-1β. First, Hed enhanced the secretion of CXCL10, IL-8 and IL-6 and IL-3 inflammatory factors, and thus further confirmed that Hed treatment suppressed the secretion of these factors in RA-FLS cells upon treatment with IL-1β, which was similar to treatment with tofacitinib (Figure 2).

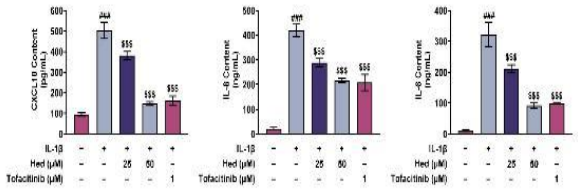

Figure 2: Hed suppressed the inflammation of RA fibroblast-like synovial cells. Data are presented as mean ± SD. $^{\#\#\#}P$ < 0.001, IL-1β vs control; $^{\&\&\&}p$<0.001, IL-1β+Hed vs IL-1β

Hed inhibit the migration and angiogenesis of RA fibroblast-like synovial cells

Figure 3 shows that Hed treatment inhibited the motility of IL-1β-induced RA-FLS cells. Hed decreased the number of migration (up) or invasion (down) cells. Hed treatment suppressed the angiogenesis of RA-FLS cells (Figure 3 B), and decreased VEGF secretion in RA-FLS cells (Figure 3 C). Thus, Hed suppressed the migration and angiogenesis of RA-FLS cells.

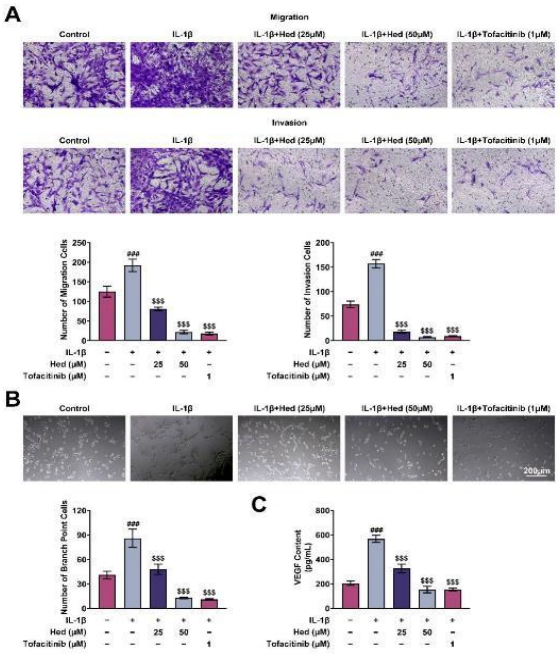

Figure 3: Hed inhibited the migration as well as angiogenesis of RA fibroblast-like synovial cells. (A) Effect of Hed on the migration and invasion of RA-FLS cells treated with or without IL-1β. (B) Effect of Hed on angiogenesis of RA-FLS. Tofacitinib treatment (1 μM) was the positive control. (C) Secretion of VEGF in RA-FLS cells upon the indicated treatment. Data are presented as mean ± SD. $^{\#\#\#}P$ < 0.001, IL-1β vs control; $^{\&\&\&}p$ < 0.001, IL-1β+Hed vs IL-1β

Hed inhibited MAPK pathway in RA fibroblast-like synovial cells

IL-1β increased the phosphorylation levels of ERK, JNK, and p38, 3 key regulators of MAPK pathway (Figure 4). Interestingly, Hed treatment decreased the phosphorylation levels of ERK, JNK, and p38, 3 key regulators of MAPK pathway in IL-1β-induced RA-FLS cells (Figure 4). Tofacitinib treatment showed similar effects (Figure 4). Therefore, Hed suppressed the MAPK pathway in RA-FLS cells.

DISCUSSION

Rheumatoid arthritis (RA) is a disease closely related to the environment and neuropsychiatric state [3]. Its pathological basis is the inflammation of synovoid cells. RA can be treated by palliative measures and drug treatment to reduce symptoms. Synovitis caused by RA is a chronic inflammation of the synovium of the joint, accompanied by angiogenesis [8]. The final treatment is mainly by medication and surgery, which can cause damage to the synovium, resulting in deformity of the joints [8].

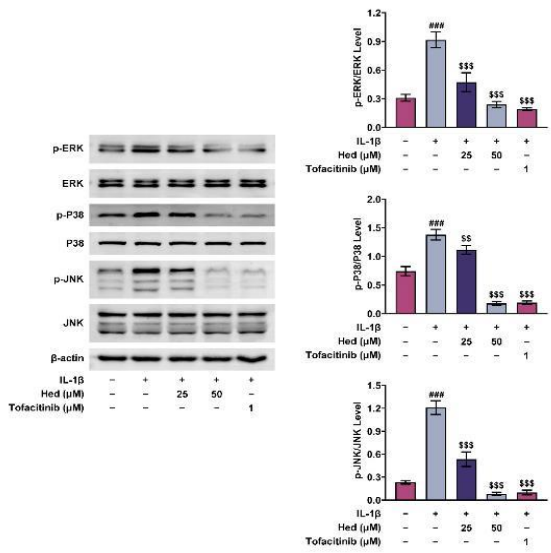

Figure 4: Hed suppressed MAPK pathway in RA fibroblast-like synovial cells. Data are shown as mean ± SD. $^{###}P < 0.001$, IL-1β vs control. &&$p < 0.01$, $^{&&&}p < 0.001$, IL-1β+Hed vs IL-1β

Drug treatment of RA mainly consists of non-steroidal anti-inflammatory drugs, but glucocorticoids may also be used [8]. However, in order to better treat RA, new therapeutic agents need to be developed. Synovial angiogenesis may exacerbate inflammation by increasing plasma extravasation. Therefore, a useful strategy to combat RA is by suppressing angiogenesis. In the present study, Hed suppressed the progression of RA by suppressing angiogenesis. Therefore, Hed is a potentially suitable drug for RA.

Hed is a triterpenoid saponin that inhibits the expressions of iNOS and NF-κB, and protects PC12 cells from corticosterone-induced damage. In addition, Hed is a potential anti-osteoarthritis drug, which significantly reduces IL-1β-induced apoptosis in C28/I2 cells [15]. Hed has been used as anti-hyperlipidemia drugs, and it significantly improves hemorheological characteristics in experimental hyperlipidemia rats [16]. These data therefore provide the evidence that Hed may suppress RA progression.

Synovial angiogenesis may exacerbate inflammation [5]. Therefore, targeted angiogenesis may be considered as a new treatment option for RA [5]. Previous studies have shown that MAPK pathway mediates the angiogenesis and inflammation of cells [17]. Therefore, Hed inhibited the proliferation, angiogenesis and inflammation of fibroblastic synovial cells in rheumatoid arthritis by inhibiting MAPK pathway, was confirmed by the data from the results from the present study.

Activation of MAPK pathway is responsible for RA, a typical feature of chronic synovitis, and can induce cytoplasmic protein phosphorylation in synoviocytes through this pathway, to phosphorylate transcription factors and nuclear proteins such as c-Fos, c-Jun, AP-1 and NF-κB, thus promoting cell proliferation and activation. p38 signaling pathway is the core mechanism mediating synovial inflammation of RA [18]. It can also be activated by cytokines. After activation, cytokines are produced to promote inflammatory response, thus prolonging joint inflammation [18].

A large amount of phosphorylated p38 was measured in T cells of RA synovial tissue. Phosphorylated p38 MAPK was also shown in synovial endothelial cells, which regulate the expression of genes encoding cell adhesion molecules [18]. After the activation of p38 MAPK, chemokines, MMP and signaling enzymes may be overexpressed in synovial tissues [18].

CONCLUSION

Hed inhibits the proliferation, angiogenesis and inflammation of fibroblastic synovial cells in rheumatoid arthritis by inhibiting MAPK pathway, and therefore, could potentially serve as a drug for RA. However, *in vivo* studies on Hed are recommended to validate these findings.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ping Wang, Junli Yang, Xiaomeng Zhang designed the study and carried them out, supervised the data collection, analyzed and interpreted the data, prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

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