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Mechanisms of catalpol regulation of RASF against RA via MAPK/GBP5 signaling pathway

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Abstract

Objective: Synovial fibroblasts (RASF) in rheumatoid arthritis are important mediators of synovial inflammation and joint destruction. This study provides valuable suggestions for the prevention and treatment of RA by observing catalase intervention in RASF and exploring its possible mechanisms.

Methods: An in vitro model of interleukin (IL)-1β-induced inflammatory synovial fibroblasts was established, and cells were intervened with different concentrations of catalpol (0, 20, 40, 60, 80, 100, 120, 140 μmol/L) for 6 h. Cell activity was measured by the CCK8 method; cells were divided into a blank group, a model group, a low dose of catalpol (20 μmol/L) group, a high dose of catalpol (100 μmol/L) group, and a high dose of catalpol (100 μmol/L) group.（The cells were divided into blank group, model group, catalpol low dose group (20 μmol/L), catalpol high dose group (100 μmol/L) and positive control group (methotrexate 100 nmol/L), and treated with different concentrations of catalpol (0 μmol/L, 20 μmol/L, 100 μmol/L) and

methotrexate (100 nmol/L) for 2 h. The cells were then stimulated with IL-1β (20 ng) for 6 h. The cell supernatants were assayed by enzyme-linked immunosorbent assay (ELISA). The cell supernatant TNF-α, IL-6 and IL-17 contents were measured by ELISA kit; the protein expression levels of p-p38/p38, p-ERK/ERK, p-JNK/JNK and GBP5 in the cells were detected by protein immunoblotting (Western blotting).

Result: Compared with the blank group, the levels of TNF-α, IL-6, IL-17 and the protein expression of p-p38/p38, p-ERK/ERK, p-JNK/JNK, GBP5 were increased in the model group cells ($P < 0.05$); compared with the model group, the catalase low and high groups (concentrations of 20 μmol/L and 100 μmol/L, respectively) and the positive control Cells (methotrexate 100 nmol/L) showed decreased TNF-α, IL-6, IL-17 content and protein expression of p-p38/p38, p-ERK/ERK, p-JNK/JNK, GBP5 ($P < 0.05$). Compared with the blank group, the cell activities of the model group, catalpol low dose group (10 μmol/L) and positive control group were decreased, while the catalpol high dose group (100 μmol/L) had increased cell activities.

Conclusion: The results of in vitro cellular assays suggest that catalpol can inhibit inflammatory RASF and may be related to the inhibition of MAPK/GBP5 signaling-mediated pro-inflammatory cytokine release, and that catalpol has potential therapeutic value in RA.

Keywords: Catalponol; rheumatoid arthritis; synovial fibroblasts.

Rheumatoid arthritis (RA) is one of the most common chronic inflammatory diseases, starting with abnormal and uncontrolled synovial inflammation, which can lead to damage to cartilage and bone in the joints, resulting in joint dysfunction and even disability **[1]**. Recurrent attacks of the disease cause great pain and suffering to patients **[2]**. Therefore, early intervention of RA synovial inflammation is a key measure in determining the treatment and prognosis of the disease **[3]**. Western medicine has no effective treatment for RA, but mainly treats symptoms symptomatically and uses hormones and immunosuppressants when there is organ involvement to control and delay the progression of tissue and organ damage caused by

immune inflammatory reactions and secondary infections, but its clinical use is severely limited by side effects such as infections **[4-5]**. Chinese medicine, on the other hand, is advantageous in improving the symptoms and signs of RA patients, avoiding adverse drug reactions, improving quality of life and reducing recurrence.

It is sweet in taste and warm in nature, and is mainly attributed to the liver and kidney meridians, with the effect of nourishing the Yin, tonifying the blood, benefiting the essence and filling the marrow. It is used to nourish the Yin and nourish the Blood, and to benefit the essence and blood. The Compendium of Materia Medica: "Fills the bone marrow, grows the muscles, and generates essence and blood" confirms the beneficial effects of Shu Di Huang on muscles and tendons. At present, Shu Di Huang is widely used in clinical practice for the treatment of inflammatory diseases **[6]**. Catalpol is a cyclic enol ether glycoside extracted from Radix et Rhizoma Dioscoreae, and the 2015 edition of the Chinese Pharmacopoeia specifies catalpol as an indicator for quality control of Radix et Rhizoma Dioscoreae **[7]**. The pharmacological effects of catalpol include anti-apoptotic and anti-inflammatory properties, but the effects on synovitis and RA have been less studied **[8]**. Therefore, the potential therapeutic value of catalpol for RA was investigated through this study.

1 Materials and methods

1.1.materials

Human rheumatoid synovial fibroblast-like cell line MH7A was provided by Mingzhou Biobank (No.336116). p-p38/p38、p-ERK/ERK、p-JNK/JNK、GBP5、β-Actin antibodies、HRP-labeled goat anti-rabbit IgG 、 RIPA protein lysate (medium) 、 protease inhibitor 、 phosphatase inhibitor (Biyuntian, China); interleukin (IL)-6, IL-17, tumor necrosis factor (TNF)-α human ELISA kit (Biyuntian, China); RPMI-17, tumor necrosis factor (TNF)-α human ELISA kit (Biyuntian, China). (China); interleukin (IL)-6, IL-17, tumour necrosis factor (TNF)-α human ELISA kit (Biyuntian, China); RPMI-1640 medium (Hyclone, USA); 10% fetal bovine serum, penicillin/streptomycin (1:100, Biyuntian, China); catalase, methotrexate (MCE, China).

1.2. Methods

1.2.1. MH7A cell culture and grouping: the cells were incubated at 37 ℃ and 5% CO_2 in the culture medium containing 10% bovine serum and double antibody. The cells were divided into blank group, model group and Catalpol low dose group (10 μMol/L) group, high dose of Catalpol (100 μMol/L) group and positive control group (methotrexate 100 nmol/L) group were treated with different concentrations of Catalpol (20 μmol/L 、 100 μMol/L) and methotrexate (100 nmol/L) for 2h, and then treated with IL-1β(20ng) stimulate the cells for 6h and carry out subsequent detection.

1.2.2. CCK8 method: 1×10^6 cells/ml density: inoculate MH7A cells into 96 well plates and culture them overnight. When the cells grow to 80%, use different concentrations of Catalpol (0, 20, 40, 60, 80, 100, 120, 140 μMol/L) for 6h, and then CCK8 was used to detect the cell viability according to the manufacturer's instructions.

1.2.3. ELISA method: 1×10^6 cells/ml density: MH7A cells were inoculated into 24 well plates and cultured overnight. When the cells grew to 80%, different concentrations of Catalpol (20 μMol/L、 100 μMol/L) and methotrexate (100 nmol/L) for 2h, and then treated with IL-1 β (20ng) stimulate the cells for 6h, collect the cell culture supernatant, and use a commercial ELISA kit to detect TNF in the cell culture supernatant according to the manufacturer's instructions- α、The contents of IL-6 and IL-17.

1.2.4. Western blotting method: according to the instructions, extract the total protein from Ripa lysate and measure the protein content by BCA method; The protein samples were transferred to PVDF membrane by SDS-PAGE electrophoresis; Sealed with 5% skimmed milk powder; Incubate with antibodies p-p38/p38, p-ERK/ERK, p-JNK/JNK, gbp5 and GAPDH respectively, rinse with tbst for 5 times, and react with antibody coupled with horseradish peroxidase after 5 minutes each time. Rinse the membrane with tbst for 10 times, develop and detect it with ECL chemiluminescence (millipore, Ma), and analyze the gray value with ImageJ.

1.2.5. statistical method: All data were calculated using SPSS 26.0 software and then plotted by GraphPad Prism 8.0 software. Measures were expressed as mean ± standard deviation ($\bar{X}$

± S), one-way ANOVA was used for multiple group comparisons, and LSD-t test was used for two-way comparisons between groups, with differences considered statistically significant at $P < 0.05$.

2. Results

2.1 Effect of catalpol on the activity of MH7A cells: MH7A cells were treated with different concentrations (0, 20, 40, 60, 80, 100, 120 and 140 μmol/L) of catalpol for 6 h. The results of the cell viability assay showed that the cell viability decreased significantly ($P < 0.05$) when the concentration reached 120 μmol/L compared with 0 μmol/L; the cell viability was not statistically significant ($P > 0.05$) when compared with 0 μ mol/L, and at concentrations of 20 μmol/L, 40 μmol/L and 60 μmol/L, the differences were not statistically significant ($P > 0.05$).

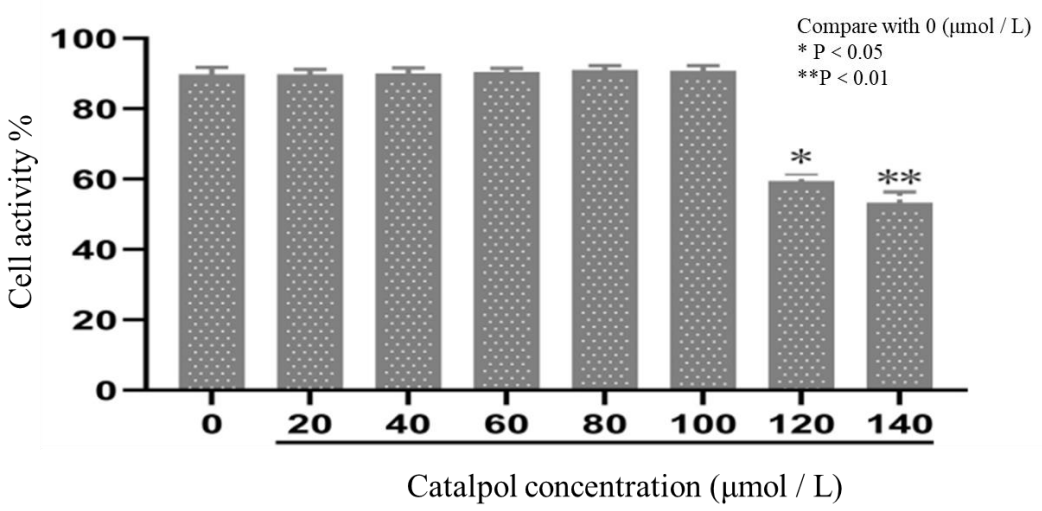

Fig.1.Effect of different concentrations of catalpol on the activity of MH7A cells

2.2. Effect of catalpol on inflammatory factors in each group of cells: The levels of pro-inflammatory cytokines TNF-α, IL-6 and IL-17 in the supernatants of cell cultures of each group were measured by ELISA. The results showed that the levels of pro-inflammatory cytokines TNF-α, IL-6 and IL-17 in the supernatants of cell cultures of the model group were significantly increased compared with the blank group ($P < 0.05$); compared with the model group, the The preadministration of catalpol at all doses and the positive control group inhibited the increase of pro-inflammatory cytokines TNF-α, IL-6 and IL-17 in the culture

supernatant of inflammatory MH7A cells ($P < 0.05$), more significantly in the catalpol high dose group ($P < 0.001$).

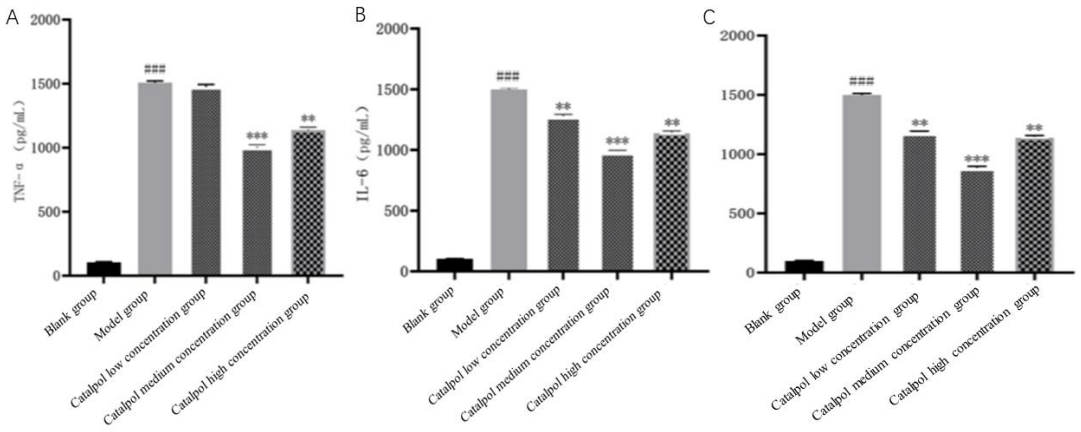

Fig.2.Effect of catalpol on the pro-inflammatory factors released by various groups of cells

(Note:MH7A cell supernatant:(A)Content of TNF-α;(B)Content of IL-6;(C)Content of IL-17.Compared to blank group ###P＜0.001； Compared to model group**P＜0.05,***P＜0.001)

2.3 Effect of catalpol on MAPK and GBP5 signalling pathways in all groups of cells: Compared with the blank group, the protein expression levels of p-ERK/ERK, p-p38/p38, p-JNK/JNK and GBP5 in inflammatory MH7A cells were significantly up-regulated ($P < 0.05$); compared with the model group, all dose groups of catalpol down-regulated the protein expression levels of p-ERK/ERK, p-p38/p38, p-JNK/JNK and GBP5 ($P < 0.05$). p-ERK/ERK, p-p38, p-JNK/JNK, and GBP5 ($P < 0.05$), and the expression levels of p-ERK/ERK, p-p38, p-JNK/JNK, and GBP5 were significantly ($P < 0.001$) downregulated in all dose groups of catalpol compared with the model group.

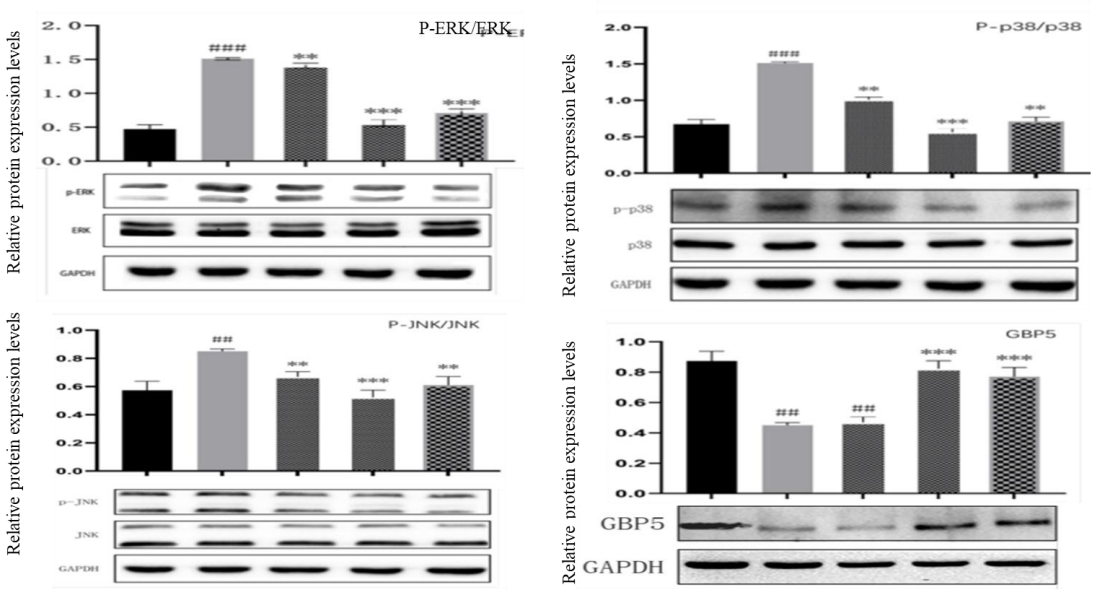

Fig.3.Effect of catalpol on MAPK and GBP5 signalling pathways in various groups of cells

(Note:Compared to blank group$^{\#\#}P < 0.05$, $^{\#\#\#}P < 0.001$； Compared to model group$^{**}P < 0.05$,$^{***}P < 0.001$)

3. discuss

Synovitis in the early stage of RA is the initial driving force to promote the development of the disease **[9]**. Because rheumatoid arthritis synovial fibroblasts (RASF) play an important role in amplifying inflammation and tissue destruction, it is not only a passive effector cell, but also has the characteristics of autonomous invasion and destruction, Therefore, the regulation of RASF is likely to improve synovial inflammatory response and improve RA symptoms **[10]**. RASF can secrete RANTES, IL-8, MCP-1 and mip-l α、 CXCL10, CXCL5, CXCL1 and other chemokines **[11]**. These chemokines recruit a variety of inflammatory cells in the blood to enter the synovium, and the inflammatory cells further secrete IL-1, IL-6 and TNF α、 GM-CSF, IL-17, IL-4 and other inflammatory factors start the inflammatory storm and cause synovial inflammation **[12]**. Moreover, RASF can directly secrete IL-6 and TNF α、 IL-1 、 IL-1 β Other inflammatory factors aggravate synovial inflammation **[13]**. Previous studies believe that the activation of RASF is closely related to the regulation of IL-6 and IL-17. MAPK (mitogen activated protein kinase) signaling pathway is the intersection of

multiple inflammatory signaling pathways κ B. Activate NF- κ B (nuclear factor of kappaB) and its nuclear translocation, which promotes the phosphorylation of downstream ERK, p38 and JNK signal pathways **[14]**. MAPK signal pathway plays an important role in the process of inflammatory response. Blocking MAPK signal is an important method for RA treatment **[15]**.

Guanylate binding proteins (Gbps) were first discovered by interferon γ Induced is a member of interferon inducible protein (ISG) **[16]**. At present, seven human derived GBP proteins (gbp1-7) **[17]** have been identified, among which guanylate binding protein 5 (gbp5) is responsible for the centralized participation in the inflammatory body activation and host defense mechanism, as well as the regulation of cell proliferation, angiogenesis and tissue invasion **[18-19]**. Previous studies have shown that gbp5 is one of the most expressed genes in RA synovium. When the expression of gbp5 in RA synovium increases, gbp5 up regulates the expression of related genes such as cytokine signaling pathway, immune response and inflammatory response, and down regulates the expression of related genes such as cell migration, apoptosis and transcription factor regulation. Gbp5 enhanced IFN- γ The anti-inflammatory effect of IL-1 β Induced reduction of inflammatory factors, indicating IFN-γ It mainly depends on gbp5 to inhibit IL-1 β Induced inflammatory mediators; At the same time, overexpression of gbp5 can directly inhibit IL-1 β Induce the production of inflammatory factors. Gbp5 overexpression significantly reduced IL-1 by lentivirus method β Induced activation of p-p38, p-JNK and p-ERK1/2 **[20-22]**. Combined with this study, it is found that different concentrations of Catalpol can inhibit the activation of MAPK and gbp5 signals in inflammatory RASF, indicating the effect of Catalpol on IL-1 β The synovitis caused by Catalpol has a certain inhibitory effect, suggesting the potential therapeutic effect of Catalpol on RA.

In conclusion, this study shows that Catalpol can inhibit the occurrence of synovitis, which may be related to inhibiting the release of pro-inflammatory cytokines mediated by MAPK and gbp5 signals. At the same time, Catalpol has potential therapeutic value for RA. However, whether Catalpol directly acts on MAPK and gbp5 signals or other signal pathways still needs to be further explored by follow-up research.

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