Effect of Tongbiling on the Synoviocyte Function in Adjuvant Arthritis Rats*

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ABSTRACT  
Objective: To study the effect of Tongbiling (TBL) on the proliferation of synovial fibroblast and interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and prostaglandin E2 (PGE2) secreted by synoviocytes in adjuvant arthritis (AA) rats. 
Methods: Synovial fibroblast was derived from culture of tissue piece. The effect of primary synoviocyte culture supernatants on the fibroblast proliferation were assayed and IL-1, TNF-α bioactivity and PGE2 content of supernatants of cultured synoviocytes were measured. 
Results: TBL could significantly inhibit the synovial fibroblast proliferation (P < 0.001), and down-regulate IL-1, TNF-α and PGE2 productions (P < 0.001); indomethacin could obviously promote the synovial fibroblast proliferation (P < 0.001). It significantly inhibited PGE2 production, but further up-regulated IL-1 and TNF-α secreted by synoviocytes (P < 0.01). 
Conclusion: The therapeutical effect of TBL on AA might be associated with its down-regulating the secretory function of synoviocyte, then restoring the abnormal proliferation of fibroblast to normal levels.

KEY WORDS  
adjuvant arthritis, synovial membrane, cell culture, fibroblast, cell proliferation, cytokine, Tongbiling

Rheumatoid arthritis (RA) is a chronic immune-mediated inflammatory disease. The early lesion of RA mainly located at synovial membrane. Various cytokines produced by synoviocytes such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) are released into synovial fluid and blood to induce the interaction between mediators which stimulate the secretion of tissue degrading enzymes. These cytokines and inflammatory mediators promote the synovial fibroblast proliferation and regeneration of synovial microvessels, which results in forming of the characteristic pannus of RA, that is thought to be a key factor not only in destruction of cartilage and subchondral bone, but also finally leads to articular interior adherence and articular deformation. (1)

Tongbiling (通痹灵, TBL) is a formula consisted of 15 ingredients of Chinese medicinal herbs (CMH) on the basis of Guizhi Shaoyao Zhimu Decoction (桂枝芍药知母汤, GSZD), a classical formula. Our previous data (2-4) had suggested that TBL has the effect of anti-inflammatory and/or immune regulatory effect, which could markedly inhibit the phalangeal destruction of animal model. In this study, the effect of TBL on the synovial fibroblast proliferation and IL-1, TNF-α and PGE2 secretion by synoviocytes in adjuvant arthritis (AA) rats were observed, and further study of its possible therapeutical mechanisms in treating RA was conducted.

METHODS

Drugs and Reagents

TBL is formulated by Cinnamomum cassia 12 g, Paeonia alba 18 g, Anemarrhena asphodeloides 12 g, Zingiber officinale 10 g, Glycyrrhiza uralensis 6 g, Ephedra sinica 6 g, Astracylodes macrocephala 12 g, Saposhnikova divaricata 12 g, Aconitum carmichaeli 6 g, Bubalus bubalis 18 g, Boswellia carterii 6 g, Commiphora myrrha 6 g, Strychnos nux-vomica 0.5 g, Scorpion 6 g, and Agkistrodon acutus 12 g; produced by the Department of Preparation of the First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine. Each tablet contains 10 g of crude drugs, lot

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1. The First Affiliated Hospital of Guangzhou University of TCM, Guangzhou (510405)
2. Shimane Medical University, Japan
Indomethacin (IM) was purchased from Shanghai 12th Pharmaceutical Factory, lot number: 950322. Freund's complete adjuvant (FCA) was provided by Sanguang Company, Japan; Lipopolysaccharide (LPS) and collagenase (type II) were purchased from Sigma Company; Trypsin was purchased from Difco Company. The above-mentioned three kinds of reagents were made up with D-Hank's buffer solution; MTT was purchased from Fluka AG Company; \[^{3}\text{H}\] prostaglandin E\(_2\) (PGE\(_2\)) RIA kit was provided by Department of Pharmacology, Chinese Academy of Medical Sciences; RPMI-1640 medium and Dulbecco’s modified Eagle’s medium (DMEM) were purchased from Gibco Co. All RPMI-1640 and DMEM media were supplemented with HEPES buffer 25mmol/L, sodium pyruvate 1 mmol/L, L-glutamine 2 mmol/L, 2-mercaptoethanol 50 μmol/L, benzylpenicillin sodium 100 u/L, streptomycin 100 mg/L, and 10% new born bovine serum and were adjusted to pH 7.2.

**Animal and Cell Line**

Sprague-Dawley rats, clean grade, male, 2–3 months old, weighing 160–210 g, and C57BL/6, female, 4–6 weeks old, weighing 18±2 g, were provided by the Department of Experimental Animals, Sun Yat-sen University of Medical Sciences. L929 cell provided by the Affiliated Zhujiang Hospital of the First Military Medical University were cultured in RPMI-1640 medium supplied with 10% new-born bovine serum.

**Apparatus**

CO\(_2\) incubator, HWO301T-VBA type, HARRIS Co., USA; centrifuge, LDZ4-OB type, Shanghai Medical Centrifuge Factory; ELISA reader, 3550 type, Bio-Rad Co., USA.

**AA Induction and Treatment**

Forty SD rats were randomly divided into four groups: TBL group, IM group, AA group and normal group. All rats except normal group were immunized by intradermal injection into the right hind paw with 0.1 ml of FCA. At 24 day after immunization, rats of the TBL group were administered daily by gastrogavage at a dose of 5 g/kg, and rats of the IM group at a dose of 2 mg/kg, rats of the AA group and the normal group were given normal saline 10 ml/kg intraperitoneally.

**Preparation of Synoviocyte Culture Supernatants**

Pertained to the referential literature,\(^{(5)}\) at the eighth week of experiment, rats were sacrificed by cervical amputation, their synovium from knees were excised at sterile condition and dispersed into single cell with sequential incubation of collagenase and trypsin. Synoviocytes were resuspended in DMEM medium and LPS (5 mg/L), then added to 24-well culture plate (1 mg/well). After incubation at 37°C in a humidified atmosphere with 5% CO\(_2\) for 48 hours, all the supernatants were collected, centrifuged, filtered and stored at −40°C before use.

**Synovial Fibroblast Separation and Culture\(^{(6)}\)**

Synovium from rat knees was excised at sterile condition and cut into tiny pieces of diameter 1–2 mm, transferred to sterile tubes, centrifuged and washed, then equally arrayed on the bottom of culture bottle, which were carefully upturned and incubated at 37°C in a humidified atmosphere with 5% CO\(_2\) for 2 hours. Next, proper volume of pre-warmed 10% fetal calf serum, the DMEM medium were mildly placed in the culture bottles and incubated further. After synovial fibroblast was grown into tissue block, remove the tiny minced tissue block, and cultured continuously. After second passage, the synovial fibroblasts were stained by Trypan blue and Giemsa, the cell purity was more than 98%, while its viability was more than 95%.

**Synovial Fibroblast Proliferation Assay\(^{(6)}\)**

Fibroblasts were resuspended in DMEM medium at a concentration of \(1 \times 10^7/L\). The cell suspension (0.1 ml) was placed in 96-well flat-bottomed culture plates and incubated for 24 hours at 37°C in a humidified atmosphere.