

The Effects of Magnetic Targeted Nano-particles upon Levels of Cytokines in Serum and Synovial Fluid of Experimental Arthritis Rats

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Abstract. The magnetic drug targeting is the use of natural or synthetic polymer material magnetic nanoparticles, solid or liquid pharmaceutical coating made of magnetic targeting of drugs. This article tripterygium targeted drug influence articular arthritis in rats and in Serum cytokines studied and discussed the pharmacology tripterygium targeted drugs. The study shows that traditional Chinese medicine in the treatment of RA Tripterygium glycosides have a significant effect, its mechanism may be contained therein Tripterygium Tripterygium glycosides tablet polyactin glucosides glycosides and other active ingredients more inflammatory cytokines in the local tissue content of inflammatory cytokine receptor upregulation or downregulation and associated with the expression of inflammatory cytokines, also may be related to immune function, correct immune abnormalities related

Introduction

The magnetic drug targeting is the use of natural or synthetic polymer materials magnetic nanoparticles coated with a solid or liquid pharmaceutical drugs made of magnetic targeting (where the diameter MM S in the range of one to several tens of microns, MN P diameter 1 nm ~ 1 000 nm), after applied to the body, under the influence of an external magnetic field can be directional movement in the body, targeting specific tumor cells or biological molecules and slowly release the drug. Ideal for targeted drug delivery system should have three elements: Positioning accumulation, control drug release, non-toxic and biodegradable[1].

Rheumatoid arthritis is a chronic, inflammatory, systemic autoimmune disease, manifested as persistent and progressive joint sinusitis, and then cause cartilage destruction and bone erosion, resulting in joint deformity, disability, severe cases eventually lost labor as well as daily living. Tripterygium is a kind of magnetic targeted drug, Qufengchushi, through the meridians, swelling and pain and other effects, have long been used to treat rheumatoid arthritis, has a large number of reported drug treatment of rheumatoid arthritis significantly, but rigorous clinical randomized controlled study little and there is no impact on quality of life in patients with rheumatoid arthritis reported, it is necessary to tripterygium treatment of rheumatoid arthritis and systematic evaluation of quality of life. We chose slow-acting drugs commonly used in clinical methotrexate (MTX) as a control, clinical efficacy and quality of life for rheumatoid arthritis patients tripterygium impact [2].

Materials and Methods

Material. Animals SPF 40 male SD rats, weighing (160 ± 10) g.

Reagents. Triptolide, Freund's complete adjuvant; IL-6, IL-10, TNFαELISA kit, 4-phenylazo phenol, isocyanate triethoxysilane; rat HMGB1ELISA kits.

Experimental Methods. First adaptive feeding one week, then 40 matches by weight randomly divided into control group and model control group methotrexate (MTX) 3.0mg / kg group Tripterygium glycosides tablet low and high dose groups (5,10mg / kg), n = 8. One group served as normal controls, and the rest used for experiments. Right toe after each rat by intradermal injection

(sc) 0.1 mL Freund's complete adjuvant to inflammation, along the back and base of the tail section of rats within five intradermal injection, every point 0.05ml, 15d minute after two intradermal injections excited. The control group received 9.0g / L for injection with sodium chloride injection. 30d after initial immunization reference arthritis index score to assess the effect of the model replicate the model to replicate the success of the rats were divided into two groups of eight.

0.1 g of the target material into the $4.5 \sim 5.3 \times 10^{-6}$ mol / L triptolide nanoparticle suspension was stirred for two days at 450 nm blue light, then wash with plenty of alcohol, until the liquid becomes colorless. The solid sample with 100ml / L of propylene glycol formulated as injection solution, wherein the solid sample containing 200 μ g / ml.

Coverage. Rats say quality, with 20ml / L sodium pentobarbital 2.5ml / kg intraperitoneal anesthesia, abdominal aortic blood, routine separation of serum to detect cytokines; rat fixed overhead position, open the knee joint cavity, with 9.0g / L sodium chloride injection 1ml flushing joint cavity, articular fluid was collected for detecting cytokines.

The rats in each group after the last administration the first two days with freshly by 3% pentobarbital 40mg / kg anesthesia, supine fixed on clean bench, abdominal aortic blood, blood samples at 4 °C, 3000r / min , 15min, separation of serum, set -20 °C to preserve, to prepare for HMGB1 and IL-17 detection.

Arthritis index. Arthritis Index (arthritisindex, AI) score method was evaluated with reference to five ratings [3], the joint lesions in rats accumulated points calculated arthritis index 0: no arthritis; 1 point: small toe joints slightly swelling; 2 points: the little toe joint and foot swelling; 3: paw swelling following ankle; 4 points: including the ankle joint swelling, including all the points accumulated up each joint, a maximum of 16 minutes for each animal.

The outer periphery of HMGB1 and IL-17 Determination. The outer periphery of HMGB1 and IL-17 content was determined by enzyme-linked immunosorbent assay to detect specific steps were measured according to kit instructions.

Statistical methods. The results are as $\bar{x} \pm s$, SPSS17.0 statistical software for statistical analysis and processing, using single factor analysis of variance between two groups were compared using q test $P < 0.05$ considered statistically significant.

Results and discussion

Tripterygium because Qufengchushi, through the meridians, swelling and pain and other effects, have long been used to treat rheumatoid arthritis, has a large number of reported drug treatment of rheumatoid arthritis significantly, but rigorous clinical few randomized controlled trials, and no effect of rheumatoid arthritis patients quality of life for the report, it is necessary to tripterygium treatment of rheumatoid arthritis and systematic evaluation of quality of life [3]. We chose slow-acting drugs commonly used in clinical methotrexate (MTX) as a control, clinical efficacy and quality of life for rheumatoid arthritis patients tripterygium impact.

AI in rats. No 7 days after immunization of rats AI significant difference on day 28 after immunization methotrexate group and Tripterygium glycosides tablet AI rats in high dose group was significantly lower than those in the control group ($P < 0.01$), which MTX group to reduce the most obvious, but there was no statistical difference between the two [4] (shown in Table 1) .

Rheumatoid arthritis is a chronic inflammatory immune damage caused by disease, HMGB1 as an important late inflammatory factor may be damaged or necrotic cells release a large number of recent studies have found that high expression of RA synovial fluid and synovial tissue of patients HMGB1 and also it plays an important role in the development of RA, HMGB1 by RAGE receptor pathway, promote chemotaxis by activating NF- κ B, to induce an inflammatory response, but also promote the secretion of IL-6, etc. in order to increase the inflammatory response [4].

Table 1 Wilforoside index change on arthritis

Groups	Dose/(mg.kg)	7 days after immunization	28 days after immunization
Model control	\	6.3±2.4	8.9±2.46
Methotrexate	3.5	4.3±1.8	4.1±1.4
Tripterygium glycosides	5.3	5.2±2.1	6.3±1.7

Determination of serum. Serum HMGB1 rat serum HMGB1 content IL-17 content in rats by ELISA, the results show: the model control group was significantly higher than the control group ($P < 0.01$), methotrexate group and Tripterygium glycosides rat serum HMGB1 content piece high dose group were significantly lower than those in the control group ($P < 0.01$), but there was no significant difference between the two; serum IL-17 content display: model control group serum IL-17 content than the control group were significantly increased ($P < 0.05$) [5], methotrexate group and Tripterygium glycosides tablet high-dose group were significantly lower than those in the control group ($P < 0.05$), but there was no significant difference between the two (shown in Table 2).

Table2 Wilforoside upon Arthritis Rats serum HMGB1, IL-17 content of influence

Groups	Dose/(mg.kg)	HMGB1/(ug/L)	IL-17/(ng/L)
Blank group	\	4.4±2.4	108.9±7.46
Model control	\	7.1±1.2	221.3±5.76
Methotrexate	3.5	6.3±1.8	155.6±6.5
Tripterygium glycosides	5.3	7.2±2.1	202.8±9.6

IL-17 acts on osteoblasts, increased expression of RANK ligand, which stimulate osteoclast precursors develop into mature osteoclasts, it can directly stimulate mature osteoclasts play a role, and therefore IL-17 inflammation persistent cell infiltration and induction of tissue damage moiety having a more direct role[5], is one of bone resorption and bone destruction of the main cytokines. In addition, the immune status of patients with RA presenting IL-17 Th1 cell polarization and cell level changes in the process of immune and inflammatory immune status is very complex, HMGB1 relations and other immune-mediated cell number and function in the disease state under effector molecules of inflammation, is attracting attention studies have shown, HMGB1 expression was positively correlated with the level of IL-17, indicating HMGB1 plays an important role in regulating cell differentiation IL-17[4].

Summary

The results showed that, CIA model in HMGB1 serum and IL-17 were significantly increased, suggesting that HMGB1 as a proinflammatory molecule in addition to the direct participation of inflammatory injury, but may also play a regulatory role for IL-17 other immune cells, thereby increasing the inflammatory damage according to The results of this study, the authors believe that IL-17 in the pathogenesis of rat CIA model secrete high levels of inflammatory cytokines IL-17, which in turn encouraged other inflammatory cytokines (such as IL-1, IL-6, TNF-) release, by somehow lead to monocyte-macrophage cells actively secreted late inflammatory factor HMGB1, so that the loss caused due to inflammation passive cells released HMGB1 already exist to further accumulation of this study showed that traditional Chinese medicine in the treatment of RA

Tripterygium glycosides have a significant effect. The mechanism may be adjusted up or down which affect the active ingredients contained in Tripterygium glycosides tablet. Tripterygium glycosides tablet polyactin more glycosides content of inflammatory cytokines in the local tissue inflammatory cytokine receptors as well. Expression of inflammatory cytokines, also may be related to immune function, correcting immune abnormalities associated. HMGB1 overexpression can stimulate synovial macrophages secrete IL-6, IL-1[6], TNF- and other inflammatory molecules that cause arthritis clinical symptoms, and Tripterygium glycosides tablet can reduce HMGB1 expression and IL-17, so that HMGB1 and IL-17 levels in tissues close to or reach the normal standard, and thus play a role in the treatment of RA, while HMGB1 and cytokines. Its mechanism of interaction of IL-17 in inflammatory response requires further studies.

In short, as so far only exists in a large number of RA in T cell-derived cytokines, IL-17 may be the evaluation of RA and therapy of a new immunological parameters, and closely associated with HMGB1 in Tripterygium glycosides tablet. CIA rats in the treatment process detection of HMGB1, IL-17 levels to help investigate the physiological role of pathology HMGB1 and IL-17 in the pathogenesis of rheumatoid arthritis, but also for HMGB1, in-depth study of IL-17 bound contribute to a better understanding of the mechanism of RA for RA treatment, especially to provide more scientific experimental evidence of natural medicine has unique advantages for clinical treatment of RA has opened new ideas and research[5,6].

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