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Combined treatment with low dose prednisone and escin improves the anti-arthritic effect in experimental arthritis

Yuan Du, Yanqin Song, Leiming Zhang, Menglin Zhang, Fenghua Fu *

Key Laboratory of Molecular Pharmacology and Drug Evaluation (Ministry of Education of China), School of Pharmacy, Yantai University, Yantai 264005, PR China

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ABSTRACT

The present study was aimed at investigating whether low dose oral prednisone combined with escin could inhibit the progression of adjuvant-induced arthritis (AIA) in rats. Adjuvant arthritis was induced in SD rats began day 1 for 28 days. Prednisone at doses of 2, 10 mg/kg/day alone or escin at doses of 5, 10 mg/kg/day alone, or prednisone at dose of 2 mg/kg/day with escin at doses of 5 or 10 mg/kg/day were given to different groups of rats intragastrically from day 13 to 28 respectively. Paw swelling, arthritic index, histological and radiographic changes were assessed to evaluate the anti-arthritic effect. Weight growth, spleen and thymus indexes were also calculated. Serum samples were collected for estimation of pro-inflammatory cytokines. Rats developed erosive arthritis of the hind paw when immunized with adjuvant. Prednisone 2 mg/kg combined with escin 5 or 10 mg/kg significantly inhibited the paw swelling. Histopathological and radiographic analysis showed a marked decrease of synovial inflammatory infiltration, synovial hyperplasia and bone erosion by combination therapy, which also markedly suppressed the expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). No significant changes were found in monotherapy group except prednisone 10 mg/kg group. Furthermore, combined treatment rescued some of GCs' adverse effects evidenced by increase in body weight and decrease in index of spleen compared with untreated AIA rats. In conclusion, the combination therapy possessed synergistic anti-arthritic efficacy and reduced adverse effect, which may play a role in the management of human RA.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease, characterized by synovial hyperplasia and chronic inflammation, which eventually results in joint destruction and functional disability [1]. It can rapidly progress into multisystem inflammation with joint damage thus causing pain, swelling, destruction of cartilage and bone, which could affect quality of life [2]. Rat adjuvant arthritis is a chronic and erosive type of arthritis induced by an injection of killed mycobacteria [3].

Although non-steroidal anti-inflammatory drugs (NSAIDs) mostly used now are efficient in alleviating basic symptoms, they could not stop its progression and protect tissues or joint from erosion. In addition, they can induce severe gastrointestinal damage, especially non-selective NSAIDs, such as aspirin and naproxen [4]. Biological therapies could not resolve clinical problems because of the high cost, selective efficacy and unknown results [5,6].

Glucocorticoids (GCs) have been used to treat RA for the last half century and recently [5]. In this regard, long-term therapy with GCs is often necessary to control the symptoms of RA and other rheumatic conditions, which could cause adverse risks, such as infections and osteoporosis, and their overall risk ratio is considered unfavorable [7]. To reduce the effects, use of lowest dose of GCs is recommended [5]. It is also reported that GCs emerge both anti-inflammatory and immune suppressive effects [8]. Thus, there remains a pressing need for an adequate solution.

People suffering from RA are seeking an alternative treatment. Combination therapy is expected to attain anti-arthritic efficacy compared with monotherapy, which could also reduce the occurrence of adverse drug effects [9,10].

Escin is one of the main bioactive constituents of *Aesculus hippocastanum*, which is well-reported for beneficial role in clinical therapy because of its anti-edematous, anti-inflammatory and anti-oxidative effects [11]. It has reported that escin is a potent anti-inflammatory drug with long anti-inflammatory effect and without immunosuppression [12,13].

We supposed that a better approach for treatment of RA, which would be to use multiple anti-inflammatory agents simultaneously to accomplish wider and more effective inhibition of the broad spectrum of inflammatory mediators involved. No study has already been conducted to investigate the effects of escin or its combination therapy on

* Corresponding author at: Department of Pharmacology, School of Pharmacy, Yantai University, 30 Qingquan Road, Yantai, Shandong 264005, PR China.
E-mail address: fufenghua@sohu.com (F. Fu).

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adjuvant induced arthritis (AIA). Hence, we explored the individual and combined effects of prednisone and escin in a rat model, on the development of arthritis.

2. Materials and methods

2.1. Reagents and drugs

Sodium escinate (SA), sodium salt of escin, was provided by Luye Pharmaceutical Co. Ltd. (Yantai, China). Prednisone acetate was produced by Zhejiang Xianju Pharmaceutical Co. Ltd. (Zhejiang, China). Complete Freund's adjuvant (CFA) (containing 10 mg/ml of dry, heat-killed *Mycobacterium tuberculosis*) was purchased from Chondrex Co. (USA). The other chemicals were of analytical grade.

2.2. Animals

Male Sprague–Dawley (SD) rats (weight, 180–220 g) were purchased from Shandong Luye Pharmaceutical Co. Ltd. (Yantai, China). All animals were acclimated for at least 1 week at temperature of 24 ± 1 °C and humidity of 55 ± 5%. The animals were maintained with free access to standard diet and tap water. The experiment procedures were approved by the local committee on Animal Care and Use.

2.3. Induction of adjuvant arthritis

CFA was prepared by suspending heat-killed *M. tuberculosis* in liquid paraffin at 10 mg/ml. Each rat, except the vehicle control group, adjuvant was intradermally injected into the right hind paw of rat for induced inflammation [8].

2.4. Experimental design

Twelve days after inoculation, the animals were selected and distributed into groups according to the severity of arthritis, so that each group had similar disease severity at the beginning of the treatment. Rats were divided into 8 different groups consisting seven animals in each. The treatment schedules were as follows:

Group I: Vehicle control group: Rats were administered intragastrically (i.g.) with only 0.5% sodium carboxymethylcellulose (CMC-Na) for a period of 16 days.

Group II: Adjuvant arthritic (AA) group: A single dose of 0.1 ml, 10 mg/ml of CFA was injected intradermally on day 1 and 0.5% CMC-Na (i.g.) for a period of 16 days.

Group III: Prednisone (2 mg/kg)-treated arthritic (Pred-2 mg) group: Arthritic rats were administered with prednisone (i.g.) at the dose of 2 mg/kg once in a day for a period of 16 days.

Group IV: Prednisone (10 mg/kg)-treated arthritic (Pred-10 mg) group: Arthritic rats were administered with prednisone (i.g.) at the dose of 10 mg/kg once in a day for a period of 16 days.

Group V: Escin (5 mg/kg)-treated arthritic (Escin-5 mg) group: Arthritic rats were administered with escin (i.g.) at the dose of 5 mg/kg once in a day for a period of 16 days.

Group VI: Escin (10 mg/kg)-treated arthritic (Escin-10 mg) group: Arthritic rats were administered with escin (i.g.) at the dose of 10 mg/kg once in a day for a period of 16 days.

Group VII: prednisone (2 mg/kg) + Escin (5 mg/kg)-treated arthritic (Pred-2 mg + Escin-5 mg) group: Arthritic rats were administered with prednisone (i.g.) at the dose of 2 mg/kg. After 30 min of prednisone treatment, rats were administered with escin (i.g.) at the dose of 5 mg/kg for a period of 16 days.

Group VIII: prednisone (2 mg/kg) + Escin (10 mg/kg) -treated arthritic (Pred-2 mg + Escin-10 mg) group: Arthritic rats were administered with prednisone (i.g.) at the dose of 2 mg/kg. After 30 min of prednisone treatment, rats were administered with escin (i.g.) at the dose of 10 mg/kg for a period of 16 days.

2.5. Measurement of paw swelling and weight growth

The right paw volume of each rat was measured using a water plethysmometer before CFA injection and then at day 3, 5, 7, 9, 11, 13, 15, 19, 23, 25 and 27 (as primary swelling), so as the body weight. From day 11, the left paw volume of rats was measured every two to four days for frequency until termination of the experiment (as secondary swelling).

2.6. Clinical assessment of arthritis

The severity of arthritis was assessed by three independent observers. The rats were observed periodically for the severity of joint inflammation after the second induction until sacrificed. The severity of arthritis was assessed on a scale of 0–4 with the following criteria [7]: 0 = no edema or swelling, 1 = slight edema and limited erythema, 2 = slight edema and erythema from the ankle to the tarsal bone, 3 = moderate edema and erythema from the ankle to the tarsal bone, and 4 = edema and erythema from the ankle to the entire leg. The arthritis score for each rat was the sum of severity in all 4 paws, with the highest score being 16.

2.7. Radiography

On day 28, rats were anesthetized with chloral hydrate, then placed on a radiographic box at a distance of 90 cm from the X-ray source. Radiographic analysis of normal and arthritic hind paws were performed by X-ray machine with a 40 kW exposition, 15 s of exposure time.

2.8. Pathological observation and measurements of serum cytokine

All rats were sacrificed on day 28, blood was collected from the abdominal aorta for determination of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) levels using ELISA kits according to the manufacturers' instructions. The legs and hind paws were removed and fixed overnight in 4% neutral buffered paraformaldehyde, decalcified for 7–10 days in 30% formic acid with 0.5 M trisodium citrate, and embedded in paraffin. Longitudinal sections (5 mm) were cut from the center of the ankle joint in the sagittal plane and stained with hematoxylin–eosin. Sections were examined by light microscopy for cellular infiltration, synovitis, bone erosion and structural integrity.

2.9. Index of spleen and thymus assay

On day 28, the rats were sacrificed via anesthesia, and the thymus and spleen were promptly removed and weighed. The index of thymus and spleen were expressed as the ratio (mg/g) of thymus and spleen wet weight versus body weight, respectively [2].

2.10. Statistical analysis

Data were expressed as mean ± standard error of the mean (S.D.). Clinical data for paw volume were analyzed by determining the area under the dosing curve with subsequent analysis of variance. AUC was calculated using Graphpad Prism software, where the area between the treatment days after the onset of disease to the termination day was computed. Means for each group were determined and % inhibition from arthritis controls was calculated by comparing values for treated