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Evaluation of the effect of andrographolide and methotrexate combined therapy in complete Freund's adjuvant induced arthritis with reduced hepatotoxicity

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ABSTRACT

Objective: Methotrexate is one of the most widely used disease-modifying anti-rheumatic drugs. The hepatotoxicity of methotrexate resulted in poor compliance with therapy. The current study was designed to analyse the combined therapy of andrographolide (AD) and methotrexate (MTX) for complete Freund's adjuvant (CFA)-induced arthritis, focusing on hepatoprotective effects, oxidative stress and arthritic-related cytokines.

Method: Wistar rats were injected with CFA into the right hind paw. Ten days post-CFA injection, the Wistar rats were administered with 1% CMC-Na solution, methotrexate (2 mg/kg/week), AD (50 mg/kg/d) and combined therapy for 35 days. The anti-arthritic effect was assessed by paw volume, X-ray and serum tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β levels. Serum samples were also analysed for glutamic oxaloacetic transaminases (GOT), serum glutamic pyruvic transaminase (GPT), alkaline phosphatase (AKP) and lactate dehydrogenase (LDH). Liver tissue samples were used to examine the cellular antioxidant defence activities using catalase activity (CAT) and GSH as well as GSH-Px and MDA. Histopathology analysis was conducted to evaluate liver damage.

Results: AD treatment strengthened the anti-arthritic capacity of MTX. AD and MTX-combined therapy additively reduced the inflammatory symptoms in CFA rats. The combined therapy of AD and MTX showed hepatoprotective effect indicated by an improvement in the serum marker, possibly due to antioxidant action and confirmed by liver histopathological changes. Furthermore, the combined therapy significantly reduced serum TNF-α, IL-6 and IL-1β levels.

Conclusions: A combined therapy of AD and methotrexate significantly alleviated MTX-induced hepatocellular injury and strengthened the anti-arthritic effect. Further clinical studies should be done to further verify the possibility of combined its clinical usage.

1. Introduction

As a systematic autoimmune disease, rheumatoid arthritis (RA) is characterized by synovial joint inflammation, articular tissue destruction and joint deformities that ultimately lead to substantial disability [1]. Therefore, it is crucially important to find therapy strategies to control the symptoms and postpone the occurrence of articular tissues destruction and joint deformities [2]. Methotrexate (MTX) is one of the most widely used disease-modifying anti-rheumatic drugs (DMARDs), which might hinder DNA and RNA synthesis [1]. The precise mechanism of low-dose MTX that regulates inflammation for rheumatoid arthritis is unknown. The anti-inflammatory effect of MTX may be mediated by adenosine [3], suppression of lymphocyte proliferation and inhibition of inflammatory cytokines [4]. Despite the proven effectiveness of MTX in treating RA, its propensity to induce hepatotoxicity [5] and haematological toxicity has restricted its clinical use. Nearly 30% of RA patients cease to receive treatment for drug-related side effects [4]. MTX may induce hepatotoxicity by an action encompassing oxidative damage on liver functions [6].

Andrographis paniculata is a plant that is used as an herbal medicine

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in China, Taiwan and other Southeast Asian countries to treat diseases associated with inflammation [7]. The bioactive andrographolide (AD), a labdane diterpenoid from the leaves of *Andrographis paniculata* extracts, shows a wide spectrum of biological activities, including inhibition on neoplasm metastasis, free radical damage, and inflammation [8]. Recently, a study by Li et al shows that AD confers protective effects in RA both in vivo and in vitro through inhibiting mitogen-activated protein kinase (MAPK) signalling pathways [7]. In terms of hepatoprotective effect, AD shows improved inflammatory response and diminished angiogenesis in thioacetamide-induced liver damage [8], and AD protects rat liver by restoring hepatic enzyme activity towards carbon tetrachloride treated animals [9]. AD shows a protective effect in other liver damage models, including d-galactosamine/lipopolysaccharide [10], hexachlorocyclohexane [11], nicotine [12] and arsenic [13], which may be related to the inhibition of inflammatory and oxidative response.

Hepatic toxic injury is one of the major issues with the clinical usage of MTX [1]. The chronic use of MTX among RA patients is usually accompanied by abnormal phenomena that elevate liver enzymes and increase the possibility of osteoporosis and gastrointestinal tract inflammation [1]. Combinatory therapy of MTX with a natural product may be a promising combined therapeutic regimen to achieve the best therapeutic effect and to eliminate pharmaceutical-related adverse effect.

Combining the adverse effect of long-term MTX therapy and the beneficial effect of AD on oxidative, inflammatory and liver damage, the current study aimed to evaluate the combined effect of AD and MTX for complete Freund's adjuvant-induced arthritis in rats. Additionally, our study was conducted to examine the ability of AD to weaken MTX-induced liver toxicity in complete Freund's adjuvant (CFA) arthritis rats.

2. Material and methods

2.1. Materials

Complete Freund's adjuvant (10 mg/ml) was bought from Chondrex (Redmond, WA, USA). Andrographolide (AD) and methotrexate (MTX) of 98% purity verified by HPLC (High performance liquid choromatography) were purchased from Meilun Biotechnology Co., Ltd. (Dalian, China); the YLS-7B toe volume measuring instrument was from Shandong Academy of Medical Sciences(Jinan, China).Serum glutamic oxaloacetic transaminases (GOT), serum glutamic pyruvic transaminase (GPT), alkaline phosphatase (AKP), glutathione (GSH), catalase (CAT), Malondialdehyde (MDA) and other kits for biochemical assays of liver function parameters were obtained from Jiancheng Ltd. (Nanjing, China). The bicinchoninic acid (BCA) Protein Assay Reagent kit was purchased from Solarbio Bioscience & Technology Company (Beijing, China). Tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β ELISA kits were from R&D Systems. AD was suspended in 1% carboxymethyl cellulose sodium (CMC-Na) solution. All other reagents and chemicals used were of analytical grade.

2.2. Experimental animals

Fifty male albino Wistar rats weighting 150–230 g were procured from Henan province Laboratory Animal center, China. Animals for our study were housed at 22 ± 3 °C in light-dark (12 h on/12 h off) and acclimated to their surroundings. All animals had free access to a standard pelleted diet and water. All experimental procedures were approved by the committee of Experimental Animal Administration of Zhengzhou University (Permission No. ZYXY 2017-0005).

2.3. Induction of arthritis

Rats were anaesthetized with 1.2% isoflurane and the subplantar surface was sterilized with 75% alcohol [5].The CFA rat model was established by injecting 0.1 ml complete Freund's adjuvant (0.1 ml containing 10 mg/ml Mycobacterium) (Chondrex, Redmond, WA, USA) into subplantar tissue of the right hind paw [14]. Subplantar injection of CFA produced obvious hydroncus within 24 h and progressive arthritis by day 9 after injection. Rats received treatment from day 10 until day 45 [14].

2.4. Experimental design

Fifty rats were divided equally into five groups of ten rats each.

Group I (Control): Healthy rats received vehicle (1% CMC-Na solution) by intragastric administration.

Group II (CFA): CFA rats were administered vehicle (1% CMC-Na solution) by intragastric administration.

Group III (MTX): CFA rats were administered intraperitoneally MTX (2 mg/kg/week) for 35 days.

Group IV (MTX + AD): CFA rats were administered intraperitoneally with MTX (2 mg/kg/week After 30 min of MTX administration, rats received AD (50 mg/kg/d) dissolved in 1% CMC-Na solution by intragastric administration for 35 days.

Group V (AD): CFA rats received AD (50 mg/kg/d) dissolved in 1% CMC-Na solution by intragastric administration for 35 days.

Blood samples were drawn from the abdominal aorta on day 45. Blood sera were separated (3000 r/min for 15 min) and kept at −80 °C. The liver was dissected out and cleaned with cold saline solution (0.85% sodium chloride), dried with tissue paper and weight. The tissue was minced and homogenized in a saline solution (0.85% sodium chloride) using a XHF-D high speed deconcentrator (Ningbo, China) and centrifuged (3500 r/min for 15 min). The supernatant obtained was used for analysis of malondialdehyde and antioxidant enzyme activities. The remaining liver sample was preserved in 10% buffered formalin for histopathological examination.

2.5. Arthritic assessment

2.5.1. Hind paw swelling

Paw swelling [15,16]: Swelling of the hind paw was detected with a plethysmograph to evaluate the development and severity of arthritis. Severity of arthritis was evaluated by measurement of paw volume on the 1st, 5th, 7th and 10th day. Then the volume of each rat was measured every 7 days after treatment on days 17, 24, 31, 38, and 45.

2.5.2. Live animal imaging and X-ray

On the 46th day, the animals were anaesthetized using 10% chloral hydrate (0.3 ml /100 mg, injected intraperitoneally) and were kept on a sample plate. X-rays of the ankle joint and paws were taken in the Bruker imaging station (Bruker In vivo Multispcetral FX PRO, USA). The X-ray assessment aimed to value the severity of peri-articular soft tissue swelling and joint space narrowness [17].

2.6. Serum biochemical factor evaluation

The liver function markers, such as serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT) and alkaline phosphatase (AKP) [18,19], were evaluated using commercial kits following manufacturer instructions. Serum levels TNF-α, IL-1β and IL-6 were determined by an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) [20]. Serum lactate dehydrogenase (LDH) activity was assessed with the commercial assay kit. LDH catalyses the oxidation of lactate to pyruvate which can react with 2,4-dinitrophenylhydrazine to generate brownish red pyruvic dinitrophenylhydrazone in alkaline solutions [21]. Enzyme activity was recorded at 450 nm for quantification of pyruvic acid, and activity was calculated as Unit/mg protein. All of the ELISA kits were used following manufacturer recommendations.