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Evaluation of the efficacy and safety of different Tripterygium preparations on collagen-induced arthritis in rats

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ABSTRACT

Ethnopharmacological relevance: Tripterygium preparations (TPs), a traditional Chinese Medicines extracted from *Tripterygium wilfordii* Hook f., are widely used for treatment of rheumatoid arthritis (RA). However, TPs from different Pharmaceutical factory have different efficacy and side effects for RA treatment.
Aim of the study: The purpose of the current study is to evaluate the efficacy and safety of four TPs from different Pharmaceutical factory in china on the treatment of collagen-induced arthritis (CIA) rats and provide a theoretical and experimental basis for the individualized use of TPs.
Materials and methods: The model of wistar rats of CIA was made, and the rats were perfused a stomach with four TPs for 3 weeks continuously. Then arthritis severity was determined by visual examination of the paws and histopathologic changes of joint, liver, kidney and testis were determined by hematoxylin–eosin (H&E) staining. The expression of inflammatory cytokines (IL-1β, TNF-α, IL-17 and IL-6) in the joint was analyzed by real-time PCR, and the count and motion parameters (sperm motility and progressive sperm) of sperm in cauda epididymis were assessed with computer-assisted sperm analysis (CASA) system. Routine blood tests were conducted using automated hematology analyzer, and the aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, creatinine (Cr), and blood urea nitrogen (BUN) in serum of CIA rats were measured using a UniCel DxC 880i autoanalyzer.
Results: All of tested TPs could reduce inflammatory score, histopathological arthritis severity and joint's inflammatory cytokines (IL-1β, TNF-α, IL-17 and IL-6) expression in CIA rats, however, TP-D showed stronger inhibitory effect for inflammatory score compared with other three TPs *in vivo*. All of tested TPs did not show hepatotoxicity and nephrotoxicity and also had little effect for the concentration of hemoglobin (Hb) and the count of white blood cell (WBC). Analysis of red blood cell (RBC) number showed that TP-C and TP-D could reverse lower RBC number in untreated CIA rats to normal level. Interestingly, the results showed TPs named TP-C and TP-D could decrease platelet (PLT) number which significantly increases in untreated CIA rats. Reproductive toxicity, the main side effect of TPs, assay showed that the sperm quality (density, viability, and motility) in four of TPs-treated CIA rats were decreased significantly, consistently with spermatogenic cell density reduced. However parallel analysis showed that in four TPs-treated rats, the number of sperm, motile sperm and progressive sperm were highest in TP-D group, in contrast, were lowest in TP-C group.
Conclusions: These findings suggested that four TPs showed significantly therapeutic effect on ameliorating inflammation of CIA rats, with no obvious hepatotoxicity and nephrotoxicity *in vivo*. TP-D showed advantages with its higher efficacy and less reproductive toxicity as well as increasing RBC number, decreasing PLT number in CIA treatment. Thus, in the development of individualized treatment plan for RA patients, TP-D might be considered preferentially.

Abbreviations: TPs, Tripterygium preparations; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; H&E, hematoxylin–eosin; CASA, computer-assisted sperm analysis; AST, aspartate aminotransferase; TwHF, *Tripterygium wilfordii* Hook f.; ALT, alanine aminotransferase; Cr, creatinine; BUN, blood urea nitrogen; Hb, hemoglobin; RBC, red blood cell; WBC, white blood cell; PLT, platelet

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1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that involves hyperplasia of synovial tissues and structural damage to cartilage, bone, and ligaments, affecting approximately 1% of the population (Sangha, 2000; Firestein, 2003). The cause of RA has not yet been fully elucidated by modern medical. Currently, clinical treatment drugs of RA are directly to reduce inflammatory mediators such as non-steroidal anti-inflammatory drugs (NSAIIDs), disease-modifying antirheumatic drugs (DMARDs), and biological agents (Kalden, 2002; Mikuls and Weaver, 2003). Although these drugs attain better effect for RA, many patients discontinue the above treatments because of adverse events or poor clinical response to biological agents (Kremer, 1999; Kalden, 2002). Furthermore, biologics are unlikely to be of general benefit in the developing world because of the financial constraints, and the relatively high medical care costs for RA restrict the application of these drugs in the developing world (Yen, 2006).

Tripterygium wilfordii Hook f. (TwHF) is one of effective Traditional Chinese Medicines in the treatment of RA (Lipsky and Tao, 1997). TwHF, also known as yellow vine wood, *Gelsemium elegans*, vegetable insecticide and red medicine, belongs to the Celastraceae Tripterygium plant (Tao et al., 1991). Anti-inflammatory and immunosuppressive compounds extracted from TwHF have been used for the treatment of a wide spectrum of autoimmune and inflammatory diseases, including RA, systemic lupus erythematosus and skin diseases in China for many years (Chen, 2001; Tao et al., 2001; Qiu and Kao, 2003; Zhang et al., 2010). Patients treated with the decoction of TwHF appeared to experience therapeutic benefit, but frequently developed adverse effects and, occasionally, severe toxicity (Canter et al., 2006). Subsequently, efforts were made to improve the extraction procedure in order to minimize toxicity and maximize therapeutic benefit (Tao and Lipsky, 2000; Ma et al., 2007). As a result, different Tripterygium preparations (TPs) were developed and used in the clinical treatment now (Xue et al., 2010). These TPs contain active extractors of TwHF. Tripterygium glycosides is main active extractor in TPs and dominates efficacy in clinical application for treatment of patients with a variety of inflammatory and autoimmune diseases for its immunomodulatory and anti-inflammatory effect (Hannington-Kiff, 1990; Chen, 2001; Xue et al., 2010). Until now, reports coming from long-term clinical practice and study have shown that TPs can reduce or replace corticosteroids and (or)-steroidal anti-inflammatory drugs for RA treatment, which possesses obvious advantages of higher efficiency and relatively lower medical care costs (Lipsky and Tao, 1997; Pyatt et al., 2000; Tao et al., 2002; Canter et al., 2006; Zhang et al., 2010; Bao and Dai, 2011). Therefore, TPs have been attracted more and more attention and widely used for clinical treatment not only in China but also in the world (Pyatt et al., 2000; Bao and Dai, 2011; Wan et al., 2014). Patients treated with TPs appeared to experience therapeutic benefit, but frequently developed some adverse effects (Sun et al., 2001; Canter et al., 2006; Bao and Dai, 2011). These side effects have plagued TPs application (Zhen et al., 1995; Gu et al., 2001; Bai and Shi, 2002; Li et al., 2009; Zhang et al., 2012). To improve efficacy and reduce side effects, more sophisticated extraction techniques are used to prepare higher purity TPs (Tao and Lipsky, 2000; Ma et al., 2007). As a result, different TPs are developed in China (Xue et al., 2010). However, so far there is no study to compare two or more different TPs in efficacy and side effects simultaneously, which is very important for not only objectively evaluating TPs clinical efficacy and side effects, but also developing individual treatment for patients.

In this study, we evaluated the efficacy and safety of four of TPs (TP-A, TP-B, TP-C and TP-D) from different Pharmaceutical factory by parallel analysis using CIA rats. TP-A, TP-B and TP-C are *Tripterygium wilfordii* multiglycoside (GTW), which is a stable glycoside extracted from TwHF, produced by Hunan Xieli, Shanghai Fuhua, and Zhejiang De Ende Pharmaceutical Co. Ltd. in china, respectively, and they are commercially available as tablets. TP-D is a new Tripterygium preparations produced by pharmaceutical factory of Chen Liji in Guangdong and it is commercially available as capsules. Besides GTW, the effective components of TP-D contain icariin (epimedium extract), betaine (medlar extract) and total flavonoids (dodder extract) (Lin et al., 2011). We found that all of tested TPs could reduce inflammatory score, histopathological arthritis severity and joint's inflammatory cytokines expression in TPs-treated CIA rats. Interestingly, TP-D showed stronger ability for inhibiting arthritic inflammation *in vivo*. Four of tested TPs did not show obvious effect on hepatotoxicity, nephrotoxicity, and also have little effect for the concentration of hemoglobin (Hb) and number of white blood cell (WBC) in CIA rats. However, both of TP-C and TP-D could reverse lower red blood cell (RBC) number in untreated-CIA rats to normal level in treated-CIA rats; TP-C and TP-D also decreased platelet (PLT) number which significantly increased in untreated CIA rats. Moreover, analysis of reproductive toxicity showed that the sperm quality (density, viability, and motility) in four of TPs-treated CIA rats were significantly decreased, consistently with spermatogenic cell density reduced. Furthermore, the number of sperm, motile sperm and progressive sperm were significantly higher in TP-D-treated CIA rats compared with other three TPs treated CIA rats, suggesting that TP-D showed lower reproductive toxicity despite of four tested TPs all decreased fertility in male rats, thus, TP-D might be beneficial for RA treatment characterized with higher efficacy and lower reproductive toxicity.

2. Materials and methods

2.1. Experimental animals

Weighing 180 ± 20 g, male, wistar rats were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Science. Mice were maintained under pathogen-free conditions. All animal experiments were conducted in accordance with guidelines and approved by the Animal Care and Use Committee of Shanghai Jiaotong University School of Medicine (2013028).

2.2. Tripterygium preparations (TPs)

TPs used in this study are showed in Table 1. TPs were dispensed with deionized water (H_2O) and administered into rats by oral gavage. The control groups were treated with H_2O only.

2.3. Establishment and treatment of collagen-induced arthritic (CIA) rats

48 Wistar rats were divided into 6 groups randomly, namely normal group, model group, TP-A group, TP-B group, TP-C group, TP-D group, each group had 8 rats. CIA was induced as described previously (Zhang et al., 2013). Briefly, Tail root of each male wistar rats were injected intradermally with 240 μg of chicken type II collagen (Chondrex, Redmond, WA, USA) in 0.05 M acetic acid emulsified in Freund's complete adjuvant. Booster injections were administered on day 7 with a total of 120 μg collagen II in Freund's incomplete adjuvant. The arthritis score was applied to evaluate paw swelling (Tanaka et al., 2006). Each paw was graded on a scale of 0–4 as follows: 0, normal, without any macroscopic signs of arthritis; 1, mild, but definite redness and swelling of the ankle or apparent redness and swelling limited to individual digits, regardless of the