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Anti-inflammatory effects and pharmacokinetics study of geniposide on rats with adjuvant arthritis

Jin-Yun Chen, Hong Wu *, Hui Li, Shun-Li Hu, Miao-miao Dai, Jian Chen

College of Pharmacy, Anhui University of Chinese Medicine, Key Laboratory of Modernized Chinese Medicine in Anhui Province, Hefei, Anhui, China

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ABSTRACT

The aim of this study was to explore the anti-inflammatory effects of Geniposide (GE), an iridoid glycoside compound extracted from *Gardenia jasminoides* Ellis (GJ) fruit in adjuvant-induced arthritis (AA) rats and its pharmacokinetic (PK) basis. AA was induced by injecting with Freund's complete adjuvant (FCA). Male SD rats were subjected to treatment with GE (30, 60 and 120 mg/kg) from day 17 to 24 after immunization. Fibroblast-like synoviocyte (FLS) proliferation was assessed by MTT. Interleukin (IL)-1, IL-6, TNF-α and IL-10 were determined using double-sandwich enzyme-linked immunosorbent assay (ELISA). Expression of p38 mitogen-activated protein kinases (p38MAPKs) related proteins in FLS was detected by Western blotting. PK profiles were simultaneously detected by ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) in AA rat plasma after oral administration of GE on day 17 after immunization. As a result, GE promoted the recovery of arthritis and inhibited the colonic inflammation damage in AA rats by decreasing the expression level of TNF-α, IL-1 and IL-6, increasing the production of IL-10 and inhibiting the expression of phospho-p38 (p-p38) related proteins in FLS. PK parameters (AUC, C_{max} and $t_{1/2}$) tended to be associated with dosage-related decreasing of efficacy index.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that leads to painful inflammation of the joints and surrounding tissues. Synovial cell hyperplasia and endothelial cell activation are early events in the pathologic process that progresses to uncontrolled inflammation and consequent cartilage and bone destruction. One of the early phases of RA [1], synovial inflammation, is resulted from abnormal activation of the fibroblast-like synovial cells (FLSs) because of their characteristics of transformed cells, losing contact inhibition *in vitro* culture [2,3]. Also, FLSs secrete large amounts of matrix metalloproteinase (MMP) and stimulate the expression of inflammatory cytokines and adhesion molecules, which directly or indirectly lead to sustained joint damage [4,5]. Pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, as well as tumor necrosis factor-alpha (TNF-α) are expressed at very high levels in synovial membrane, which are considered to be the important participants in the pathophysiology of RA. Based on the pathogenic mechanisms, specific therapeutic interventions can be designed to suppress synovial inflammation and joint destruction in rheumatoid arthritis.

In the search for new naturally occurring anti-inflammatory agents in plants, we found that *Gardenia Jasminoides* Ellis (Rubiaceae), known as “zhizi”, a notable traditional medicine, is widely used for treating rheumatism, knee pain, traumatic bleeding, and liver, stomach or nerve disorder. Its pharmacological actions such as antihypertensive, antioxidative, antiangiogenic, anti-sepsis and hypolipidaemic activities have already been demonstrated [6–9]. Apart from its indigenous uses, *Gardenia Jasminoides* (GJ) has also gained importance in modern medicine due to geniposide (GE). GE, an iridoid glycoside purified from the fruit of the herb, is the major active ingredient of GJ (structure, see Fig. 1), which possessed antiangiogenic [10], anti-inflammatory [11,12], anti-vascular injury [13] and protective effects against hepatic damage [14]. Our precious study has demonstrated the mechanisms under its anti-inflammatory and immune-regulatory effects were through inducing T helper 17 (Th17) cell immune tolerance and enhancing regulatory T (Treg) cell-mediated activities by down-regulating the expression of phosphor-c-Jun N-terminal kinase (p-JNK) [15].

p38MAPK is one of intracellular serine/threonine protein kinase superfamily members. Many studies suggested that the p38 signal pathway plays a pivotal role in the progress of considerable human diseases, specifically its involvement in the development of RA [16]. Activated p38MAPK contributes to the overexpression of pro-inflammatory cytokines, chemokines, MMPs and signaling enzymes (COX-2) in the inflamed synovium. It may also increase recruitment of other inflammatory cells leading to degradation of bone and cartilage. Blockage of p38MAPK with p38 inhibitor could partially inhibit [17] the inflammatory cascade response, decreasing the downstream inflammatory cytokine production. It tips that p38MAPK may be a therapeutic target for treating RA. Here, we evaluated the effects of GE on regulating cytokines

* Corresponding author at: College of Pharmacy, Anhui University of Chinese Medicine, Hefei 230031, China. Tel.: +86 551 6516 9230; fax: +86 551 6516 9222.
E-mail address: hongw@aliyun.com (H. Wu).

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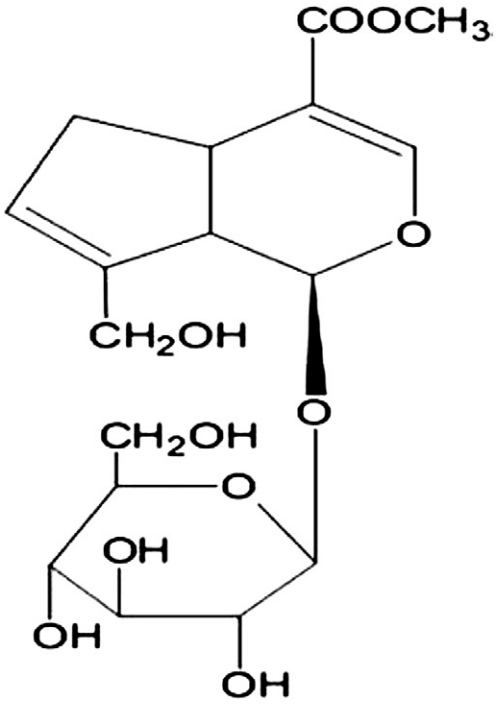

Fig. 1. Structure of Geniposide (formula $C_{17}H_{24}O_{11}$, molecular weight 404.36).

from molecule and protein level, respectively. Our results showed that GE could inhibit the progressive inflammation by inhibiting the proliferation of FLS and its secretion of pro-inflammatory cytokines and the expression of p-MKK3/6, p-p38 and p-MAPKAP2 in FLS, suggesting that GE has a significant therapeutic potential in treating inflammatory disorders.

On the other hand, since GE is now being developed as an anti-inflammation agent for treating RA, there is merit in characterizing the pharmacokinetic profiles in animals. Several analytical methods for the quantification of GE in biological samples have been reported [18–20]. However, investigation of PK values of GE in disease states has been seldom reported and the relationship between anti-inflammatory effect and pharmacokinetic study of GE on AA rats remains unclear. In this paper, we explored the effects of GE on AA rats and its relationship with PK profiles for the first time.

AA in the rat is an experimental model that shares some features with human RA [21], such as swelling, cartilage degradation, and loss of joint function. In this study, we use AA rats to mimic the acute phase in human RA and assess the effects and PK profiles after oral administration of GE. GE was extracted and purified from GJ by solvent extraction and column separation. And the structure of GE was identified by physicochemical properties and spectroscopic analysis, and the content was determined by UPLC. Sinomenine (Sin), as the positive control drug, was used for the treatment of AA animals. It was expected that the results of this study would be helpful for improving clinical therapeutic efficacy and predicting clinical effective dosage of GE.

2. Materials and methods

2.1. Chemicals and reagents

Geniposide is a yellow powder with >94.43% purity (determined by UFLC XR, Shimadzu, Japan), which was extracted and purified from GJ, as described previously [22]. The reference standards (purity >98%) of GE were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Bacillus Calmette Guérin (BCG) was obtained from Shanghai Biochemical Factory in Shanghai, China. Freund's Complete Adjuvant (FCA), lipopolysaccharide (LPS), trypsin, and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co (St. Louis, Mo). Dulbecco's Modified Eagle's Medium (DMEM), which was purchased from the Gibco Company (Los Angeles, CA), was adjusted to pH 7.2. Mouse anti-(MKK3/6, p-MKK3/6, p38, p-p38, MAPKAP2, p-MAPKAP2) monoclonal antibodies were obtained from Santa Cruz Biotechnology, Inc. β-actin was purchased from ZSGB-BIO. Rat IL-1 and IL-6 ELISA kits were supplied from Elabscience Biotechnology Co., Ltd. Rat IL-10 and TNF-α ELISA kit were from Biosource International, Inc. (Camarillo, USA). Sinomenone Hydrochloride Tablets were provided by Luyin Pharmacy Co., Ltd (Shandong, China). All the other reagents were of analytic grade and obtained from commercial sources.

2.2. Preparation of free geniposide

Free GE solution was prepared by suspending pure GE in 0.5% sodium carboxymethylcellulose (CMC-Na) with concentrations of 30, 60 and 120 mg/kg.

2.3. Animals

Male Sprague–Dawley rats (200 ± 20 g, Grade II, Certificate No.006) were purchased from the Experimental Animal Center of Anhui Medical University (Hefei, China). Animals were housed under 25 ± 1 °C and 55 ± 5% relative humidity on a 12 h light–12 h dark cycle with food and water provided *ad libitum*. Rats were acclimated in the laboratory for at least one week prior to experiment. All procedures and animal care were approved by the Committee on Ethics of Animal Experiments, Anhui University of Chinese Medicine (Hefei, China), and were conducted according to the *Guidelines for Animal Experiments* of Anhui University of Chinese Medicine.

2.4. Induction of adjuvant arthritis rats and division of treatment groups

AA model was induced by the chemical reagents method as described previously [23]. Before the onset of arthritis, forty animals with AA were randomly divided into 5 groups: three dosages of geniposide groups (30, 60, or 120 mg/kg, respectively), Sin group (positive control group, 90 mg/kg), and model group (an equal volume of CMC-Na). Additionally, eight rats used as normal control group were given an equal volume of vehicle (CMC-Na) at the same time. The animals were administered by intragastric means once a day from day 17 to day 24 after immunization.

2.5. Evaluation of arthritis and effect of GE on AA rats

From day 7 after immunization, all group rats were examined every 3 to 4 d for 3 clinical parameters: paw volume, arthritis systemic assessment and histological examination. Left hind paw volume was measured with an MK-550 volume meter (Muromachi Kikai Co., Tokyo, Japan) before immunization (basic value, day 0). Paw swelling is expressed as increasing in paw volume in milliliters calculated by subtracting the basal value from the paw volume measured at all times considered.

Arthritis systemic assessment was based on symptoms of different AA rat parts [24] (no nodule and redness equals 0, nodule and redness with one ear equals 1, nodule and redness both ears equals 2; nose part: no connective tissue swelling and redness with nose equals 0, evident connective tissue swelling and redness equals 1; tail part: no nodule and redness with tail equals 0, evident nodule and redness equals 1; paw part: 0 to 4 swelling and redness equals 0–4). The maximum value of each rat scored 8. Two of the above-mentioned assessments were repeated from day 7 to day 24 after immunization.

The rats were sacrificed on d 24 after immunization. The secondary hind paws were removed, fixed in 10% formalin, decalcified in 5% formic acid, and then embedded in paraffin. Serial paraffin sections were