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The investigation of anti-inflammatory activity of Yi Guanjian decoction by serum metabonomics approach

Sufang Shui[a,b], Xiaorong Cai[b,c], Rongqing Huang[a,b,*], Bingkun Xiao[b], Jianyun Yang[b]

[a] Anhui Medical University, Hefei 230032, China
[b] Institute of Radiation Medicine, Academy of Military Medical Science, Beijing 100850, China
[c] Guangdong Pharmaceutical University, Guangdong 510006, China

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ABSTRACT

Yi Guanjian (YGJ), one of the Chinese herbal medicines most commonly used in western countries, reported to possess significant anti-inflammatary effects that inhibit the process of inflammation. However, the mechanisms underlying its anti-inflammation effects remain largely unresolved. This study was aimed to investigate the anti-inflammatory activity of YGJ and to explore its potential anti-inflammatory mechanisms by serum metabonomics approach. An xylene-induced mouse right-ear-edema model was used as an inflammatory response *in vivo* model. Ear edema, prostaglandin E2 (PGE_2) and Tumor-Necrosis-Factor-alpha (TNF-α) were detected. Then, serum metabolic profiling was analyzed and pathway analysis performed on the biomarkers reversed after YGJ administration and further integration of metabolic networks. The results showed that YGJ alleviated ear edema and decreased serum PGE_2 and TNF-α levels. Fourteen biomarkers were screened, and the levels were all reversed to different degrees after YGJ administration. These biomarkers were mainly related to linoleic acid metabolism, taurine and hypotaurine metabolism, glyoxylate and dicarboxylate metabolism, glycine, serine and threonine metabolism and citrate cycle (TCA cycle). In metabolic networks, glycine and pyruvate were node molecules. This indicated that YGJ could significantly inhibit inflammatory response triggered by acute local stimulation and exerted anti-inflammatory activity mainly by regulating node molecules.

1. Introduction

Inflammation is a fundamental part of the immune response and is triggered by tissue damage or pathogens infection [1]. Vascular reactivity, which can induce vasodilation and an increase in vascular permeability, is central to the inflammatory process [2]. Moreover, vascular permeability factor (VPF) plays an important role in the release of inflammation mediators from blood into tissues by enhancing the vascular permeability [3]. Inflammation mediators, such as prostaglandins and Tumor-Necrosis-Factor-alpha (TNF-α) in peritoneal fluids, are triggered by tissue damage, such as burns, microbial infections, injuries and other noxious stimulus to combat infection or repair injury [4]. The anti-inflammatory agents exert their effects through various models of action. Xylene-induced mouse ear edema is often used to study inflammation [5].

Yi GuanJian, a traditional Chinese medicine, has been an effective and safe Chinese herbal formula in clinical applications for long time [6]. This formula consists of six herbals: Rehmanniae Radix (Rehmannia glutinosa Libosch), Lycii Fructus (Lycium barbarum), Angelicae Sinensis Radix (Angelica sinensis (Oliv.) Diels), Glehniae Radix (Glehnia littoralis Fr. Schmidt), Ophiopogonis Radix (Ophiopogon japonicus (L. f) KerGawl) and Toosendan Fructus (Melia toosendan Sieb. Et Zucc.), respectively. Its therapeutic functions include anti-hepatic fibrosis, antihypoxia, anti-fatigue, analgesia, anti-inflammation, anti-ulcer and resistance to sjogren's syndrome [7]. Therefore, this study focused on the anti-inflammatory mechanisms of the plant extract by investigating the animal model including xylene-induced mice ear edema.

Metabonomic analysis is an useful approach to revealing the metabolic aspects of inflammation because metabonomics comprehensively detects and quantifies the metabolite composition in an integrated biological system and its dynamic responses to the changes of both endogenous and exogenous factors [8]. Metabonomic studies on urine, serum, and tissue have become prevalent and achieved great progress. Metabonomics largely depends on

* Corresponding author at: Anhui Medical University, Hefei 230032, China.
E-mail address: rqhuang@aliyun.com (R. Huang).

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other advanced instrumental analysis, such as Fourier Transform Infrared Spectrometer (FT-IR), Liquid Chromatography-Mass Spectrometry (LC–MS), Nuclear Magnetic Resonance (NMR) spectroscopy and Gas Chromatography-Mass Spectrometry (GC–MS). GC–MS based metabonomics is a well-established and powerful technique for rapidly identifying changes in the global metabolite profiles of biological samples thanks to its complete database of mass spectra [9]. In this study, we used a GC–MS based metabonomic approach coupled with ELISA analysis to obtain serum metabolite markers associated with inflammation and to evaluate effects of Yi Guanjian on the progression of inflammatory response.

2. Materials and methods

2.1. Preparation of the extract

Yi GuanJian, a classical traditional Chinese medicine, is an aqueous extract of six herbal medicines: Rehmanniae Radix (300 g), Lycii Fructus (100 g), Angelicae Sinensis Radix (100 g), Glehniae Radix (100 g), Ophiopogonis Radix (100 g), Toosendan Fructus (50 g). The mixture was decocted three times with boiling water (1 h each time). All the decoction was then merged, filtered and concentrated under reduced pressure to fluid extract, which was dried under vacuum to obtain dry powder (300 g) and stored at 4 °C until use.

2.2. Reagents and materials

Rehmanniae Radix, Lycii Fructus, Angelicae Sinensis Radix, Glehniae Radix, Ophiopogonis Radix and Toosendan Fructus were purchased from Tongrentang Chinese Medicine (Beijing, China). 0.9% NaCl was from Shijiazhuang (Hebei, China) Siyao co., LTD. Ultrapure water was prepared by a Milli-Q water-purification system from milipore (Bedford, MA, USA). The assay kit used for prostaglandin E2 (PEG2) and Tumor-Necrosis-Factor-alpha (TNF-α) was purchased from eBioscience (Shanghai, China) Biological Technology co., LTD. Methanol (HPLC grade) was purchased from Fisher Scientific (Waltham, MA, USA). Methoxyamine, pyridine, Nmethyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) were purchased from Sigma (Shanghai, China). Aspirin was purchased from Bayer HealthCare Manufacturing S.r.l. (Beijing, China). Xylene (Analytical grade) was provided by Sinopharm Chemical Reagent (Beijing, China). Sigma 3–18k High Speed Tabletop Refrigerated Centrifuge was provided by Shanghai (Shanghai, China) Anting co., LTD. LGJ-25C freeze drier, Beijing (Beijing, China) Sihuan co., LTD. Victor X5 PerkinElmer (Shanghai, China) co., LTD.

2.3. Animals and treatment

Male Kunming (KM) mice (22–25 g) were supplied by the Experimental Animal Center, Academy of Military Medical Sciences (Beijing, China). Animal welfare and experimental procedures strictly complied with the National Institute of Health Guidelines. The animals were housed under standard laboratory conditions at a temperature of 25 ± 2 °C, relative humidity of 50–55% and 12 h light/dark cycle and acclimatized to the environment at least one week before animal experiments. After that, mice were divided into six groups (n = 8): low-(LD, 5.39 g/kg body weight), medium-(MD, 10.78 g/kg body weight), high-dose (HD, 21.56 g/kg body weight) groups of Yi Guanjian, aspirin group (ASP, 0.58 g/kg body weight), normal control group (CON) and model group (MOD). CON and MOD groups were gavaged with the same volume of pure water. Each group was given drugs or water once a day at 8:00-9:00 a.m. for 15 consecutive days. Xylene-induced ear edema in mice was initiated according to a previously described method [10]. Edema was induced by applying 20 μL xylene to the right ear of the male mice. On the last day, all the mice were administrated orally 30 min before xylene was applied. After another 1 h of xylene application, all the male mice were sacrificed by cervical dislocation and both ears were removed. Samples were removed and weighed quickly with a cork borer (7 mm in diameter). The edema degree was responded with the difference in weight between the two samples of a mouse to evaluate the effect of the extract of Yi Guanjian decoction. Edema degree(%) = $(M_R - M_L)/M_L$*100%, where, M_L = the mass of the left ear; V_L = the mass of the right ear one hour after xylene was applied to the right ear.

2.4. Serum biochemical parameters

All the blood samples were collected in heparinized tubes and centrifuged at 12000 rpm for 10 min. Serum samples were separated and stored at −80 °C for further use. Part of each serum sample was used for PGE_2 and TNF-α tests, and the other part for GC–MS metabonomics analysis. The serum samples were thawed at room temperature prior to analysis. PGE_2 and TNF-α tests were performed according to the instructions.

2.5. GC–MS sample preparation

The serum samples were thawed at room temperature prior to analysis. To start with, 1200 μL of methanol was added into 100 μL of the sample for protein-precipitation. The mixture was ultrasonically extracted for 15 min, followed by centrifugation (12000 rpm) of another 10 min. Then, 200 μL supernatant was transferred to the GC vial and evaporated to dryness under a stream of nitrogen gas. Next, 50 μL of methoxyamine pyridine solution (15 μg/mL) was added to the vial, and methoxymation was performed at 70 °C for 1 h. MSTFA (50 μL, with 1% TMCS) was added, and the silylation was performed at 70 °C for 1 h. After the addition of 100 μL of n-heptane and vortex mixing, the mixture was centrifuged for 10 min. The supernatant was transferred to the GC microvial for GC–MS analysis.

2.6. GC–MS analysis

GC–MS analysis was carried out using an SHIMADZU GCMS-2010 Plus series equipped with an Rxi–5 ms capillary column (30 m × 0.25 μm × 0.25 mm). The initial temperature was maintained at 80 °C for 5 min, and then raised to 280 °C at a rate of 4 °C/min. All the samples were injected in a split mode at 280 °C. The mass spectrometer was operated in an EI mode (70 eV), and the quadrupole was 150 °C. Mass spectra were acquired in a full scan mode with repetitive scanning from 60 *m*/*z* to 600 *m*/*z* for 1 s. The ion source temperature was 230 °C, interface temperature was 280 °C, and the solvent cut time was 4 min.

2.7. Data analysis

GC–MS data were exported in the comma delimited format (.cdf). Raw data were imported into XCMS online software and then processed with default settings to carry out baseline correction, peak discrimination and alignment, retention time correction. The data matrix was normalized to the sum of all peak areas of each sample, and then the missing value was eliminated via the 80% correction method.

Pattern recognition based on principal component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA) were accomplished using SIMCA-P V11.5 (Umetrics, Sweden) after mean-centering and autoscaling. The unbiased tool of PCA was used to detect the intrinsic clustering. Then, the supervised method of PLS-DA was employed to study the contribution of the variables in group separation, and potential metabolite markers screening