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Identification and distribution of four metabolites of geniposide in rats with adjuvant arthritis

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ABSTRACT

Geniposide (GE), also called Jasminoidin, is the major active ingredient of *Gardenia jasminoides* Ellis (GJ) fruit, which has long been used in traditional Chinese medicine (TCM). Growing evidences suggested that GE has a great potentiality for treating rheumatoid arthritis (RA). However, GE is rapidly metabolized, and we know little about its availability or metabolites in tissues. To elucidate the distribution of GE and its metabolites in tissues, three groups of adjuvant arthritis (AA) rats were given GE (33, 66 and 120 mg/kg) from days 18 to 24, and the biotransformation of GE in plasma, liver, spleen, synovium, urine and mesenteric lymph node (MLN) of rats was investigated by a novel approach named Information-Dependent Acquisition (IDA)-Mediated LC–MS/MS method. As a result, GE and its four major metabolites were detected as follows: GE, G1, G2 in plasma; GE, G2 in MLNs; only GE in liver and synovium; GE, G2, G3 and G4 in spleen; and GE, G1, G2 and G4 in urine. In total four metabolites (G1–G4) involved in the in vivo metabolism processes were identified. The results of this work have demonstrated the IDA-Mediated LC–MS/MS could screen rapidly and reliably the characterization of metabolites from iridoid compounds.

1. Introduction

RA is a chronic systemic disease of unknown etiology, which is characterized by an inflammatory process in synovium resulting in progressive destruction of cartilage and bone in affected joints [1–3]. Moreover, it is the most common inflammatory arthritis and a major cause of disability [4].

GE (Fig. 1(A)), also called Jasminoidin, is a water-soluble iridous glycoside purified from gardenia fruit which has been reported to treat hepatic and inflammatory conditions [5–9]. However, recent reports [10,11] suggested that GE causes liver toxicity, and most of the investigators believed that the transformation process of GE into aglycone genipin (Fig. 1(B)) or other metabolites was related to the liver toxicity of GE. Therefore, it is important to find out, which of the protagonist for the pharmacological effects of GE is, itself or its metabolites. AA is widely used as an experimental model, which shares some features with human RA in some pathological, histological and immunological aspects [12]. Some studies have focused on the quantification of GE in normal rat plasma and the identification of metabolites in normal rat urine with LC–MS/MS [13–15]. Our preliminary results showed that the time of GE reaching C_{max} (peak concentration) was at 1 h after oral administration with GE in AA rat plasma [16]. Han et al. found

Abbreviations: GE, geniposide; TCM, traditional Chinese medicine; RA, rheumatoid arthritis; MLN, mesenteric lymph node; AA, adjuvant arthritis; IDA, Information-Dependent Acquisition; SRM, selected reaction monitoring; ESI, electrospray ionization; EPI, enhanced product ion; SD, Sprague–Dawley; FCA, Freund's complete adjuvant; Ep, Eppendorf; MRM, the multiple reaction monitoring; TIC, total ion chromatogram; DP, declustering potential; CE, collision energies; CXP, Cell Exit Potential; XIC, extracted ion chromatogram; EP, Entrance Potential; CUR, Curtain Gas; IS, IonSpray Voltage; TEM, Temperature; Gas 1, Ion Source Gas 1; Gas 2, Ion Source Gas 2; GlucA, glucuronic acid; PK, pharmocokinetics.

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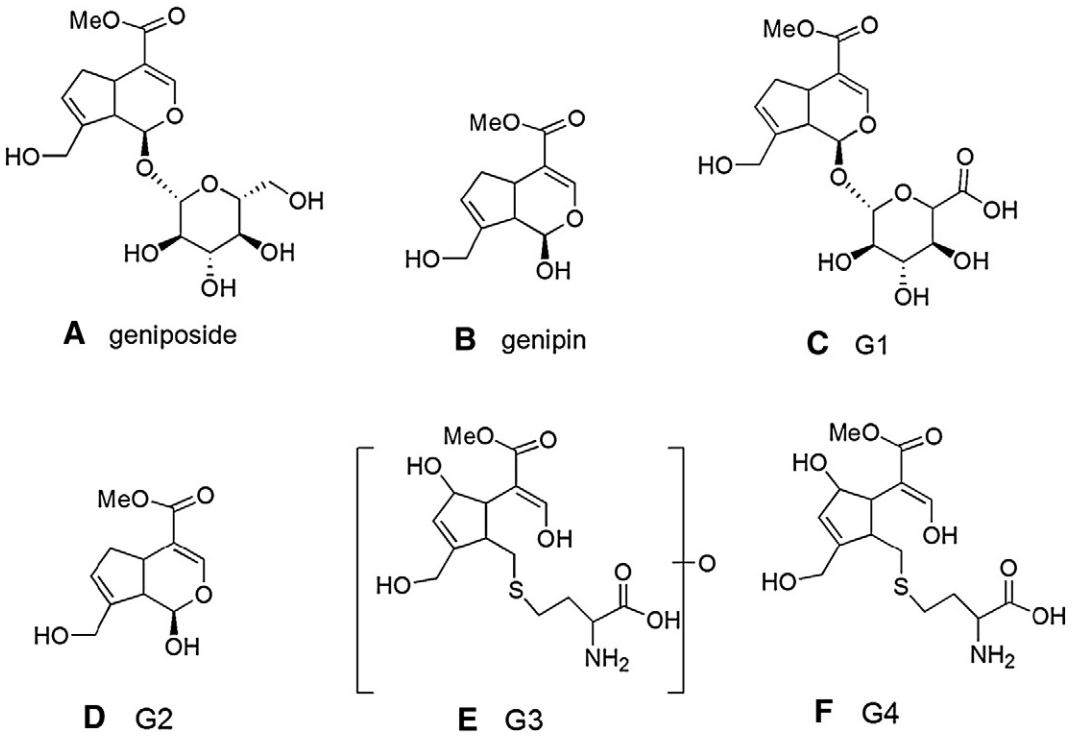

Fig. 1. Chemical structure of geniposide (A), genipin (B), metabolite G1 (C), metabolite G2 (D), metabolite G3 (E), metabolite G4 (F).

that the glucuronidation of the aglycone of GE and its ring-cleavage derivatives were the main metabolic products observed in urine sample [15]. Moreover, it was proved that GE would transform into genipin and a nitrogen-containing metabolite genipinine by intestinal bacteria in humans [17]. However, further researches in specific disease state and associated areas involving immune tissues and effector tissues have not been reported. It is helpful for further revealing the pharmacological mechanism of GE and screening new therapeutic formulas for RA to explore the metabolism of GE in immune tissues of rats with AA. Information-Dependent Acquisition (IDA)-Mediated LC–MS/MS method is an advanced approach in qualitative study of many target compounds and has been successfully used to support plasma pharmacokinetic screening programs [18]. This method presents a screening procedure based on the injection of tissue samples (after handled) in a LC–ESI-MS/MS system (AB SCIEX QTRAP® 4500). Its feasibility within a forensic toxicological setting has been demonstrated by assessing the potential and pitfalls of the IDA-Mediated LC–MS/MS screening approach [19].

2. Experimental

2.1. Materials

GE (purity > 98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The standard of genipin (purity > 98%) was supplied by Chengdu Must Bio-technology Co., Ltd. LC grade formic acid was purchased from ROE. Acetonitrile and methanol, HPLC grade, were obtained from Fisher (Shanghai, China). Deionized water was prepared by Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA). Physiological saline samples were purchased from the Tian Bao Biopharmaceutical., Ltd. Guangdong Province (brand A, plastic bag).

2.2. Apparatus

Instrumentation-MS/MS analysis was constitute of an Agilent 1290 binary pump HPLC system (Agilent technologies Inc., USA) fitted with an ACQUITY UPLC™ HSS C18 column (100 mm × 2.1 mm i.d., 1.8 μm particle size) interfaced to an AB SCIEX Triple Quad TM 4500 Mass Spectrometers (AB SCIEX, USA). An Analyst® 1.6.1 Software controlled the LC–ESI-MS/MS system and processed the data. Centrifuge (Eppendorf 5430R, Germany) was purchased from Eppendorf.

2.3. IDA-Mediated LC–MS/MS condition

2.3.1. Chromatographic conditions

The mobile phase consisted of 0.1% formic acid in water (A) and 90% formic acid in acetonitrile (B). The gradient applied was as follows: from 0 to 2 min 98% A and 2% B, from 2 to 10 min to 100% B, from 10 to 11 min to 98% A, and from 11 min 98% A and 2% B. Run time was 15 min followed by a 1 min delay prior to the next injection. Flow rate was 0.2 mL/min. Column temperature was kept at 20 °C.

2.3.2. Mass spectral conditions and IDA

Mass detection is performed in the negative ion IDA mode, a selected reaction monitoring (SRM) experiment as survey scan and the “enhanced product ion” (EPI) scan as dependent