Food and Chemical Toxicology 46 (2008) 2764–2769

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Metabolism and pharmacokinetics of genipin and geniposide in rats

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ARTICLE INFO

Article history:
Received 31 August 2007
Accepted 28 April 2008

Keywords:
Geniposide
Genipin
Gardenia jasminoides
Pharmacokinetics
Toxicity

ABSTRACT

Geniposide, an iridoid glucoside, is a major constituent in the fruits of *Gardenia jasminoides* (Gardenia fruits), a popular Chinese herb. Genipin, the aglycone of geniposide, is used to prepare blue colorants in food industry and also a crosslinking reagent for biological tissue fixation. In this study, we investigated the metabolism and pharmacokinetics of genipin and geniposide in rats. Blood samples were withdrawn via cardiopuncture and the plasma samples were assayed by HPLC method before and after hydrolysis with sulfatase and β-glucuronidase. The results indicated that after oral administration of genipin or Gardenia fruit decoction, genipin sulfate was a major metabolite in the bloodstream, whereas the parent forms of genipin and geniposide were not detected. Importantly, oral administration of 200 mg/kg of genipin resulted in a mortality of 78% (7/9) in rats.

1. Introduction

Geniposide, an iridoid glucoside, is a major constituent in the fruits of *Gardenia jasminoides* E_{LLIS} (Gardenia fruits, GF) which is a popular Chinese herb used to treat febrile diseases including edema, hepatic disorders, acute conjunctivitis and hematuria (Chang and But, 1987). GF decoction is a traditional dosage form in clinical Chinese medicine and the preparation was done by extracting the crude drugs of GF with boiling water.

It was found that intestinal bacteria in animal can transform geniposide to its aglycone genipin (structures shown in Fig. 1) (Akao et al., 1994). Besides being used to prepare a series of blue colorants in food industry, genipin is also an effective crosslinking reagent for biological tissue fixation (Fujikawa et al., 1987; Sung et al., 1998). Until now, various bioactivities of genipin and geniposide have been identified in numerous studies, including modulation on proteins (Kang et al., 1997; Kuo et al., 2004, 2005), antitumor (Hsu et al., 1997; Koo et al.,2004a; Kim et al., 2005), anti-inflammation (Koo et al., 2004b, 2006), immunosuppression (Chang et al., 2005), antithrombosis (Suzuki et al., 2001), and protection of hippocampal neurons (Yamazaki et al., 2001; Lee et al., 2006). However, most of the results have been obtained by *in vitro* studies. Based on our knowledge on the biological fates of glycosides and related aglycones, we hypothesized that the parent forms of genipin and geniposide may not enter the circulation (Hou et al., 2008). Therefore, without the knowledge of the metabolism and pharmacokinetics of genipin and geniposide in animals, it is difficult to infer the *in vivo* effects from *in vitro* activities, on which most aforementioned studies concerned.

In regard to the pharmacokinetics of geniposide, a previous study had reported simultaneous estimation of geniposide and genipin in mouse plasma after oral administration of GF decoction (Ueno et al., 2001). Another study described the pharmacokinetics of geniposide after intravenous administration (Tseng and Tsai, 2004). Recently, a study reported the pharmacokinetics of geniposide in rat serum after oral administration of Yin-Zhi-Ku decoction (Ye et al., 2006). Notwithstanding these new results, the dearth of information concerning the metabolism of genipin and geniposide *in vivo* led us to investigate the metabolism and pharmacokinetics of genipin and geniposide in rats following oral administrations of genipin and GF decoction.

2. Materials and methods

2.1. Materials

The dried fruits of *G. jasminoides* were purchased from a Chinese drugstore in Taichung and the origin was identified macroscopically and microscopically. Genipin was supplied by Challenae Bioproducts Co. Ltd. (San Jose, CA, USA). Polyethylene glycol 400 was purchased from Merck Schuchardt OHG (Hohenbrunn, Germany). Heparin was obtained from Novo Nordisk (Bagsvaerd, Denmark). Sulfatase (type H-1 from *Helix pomatia* from *Helix pomotia*, containing 14,000 units/g of sulfatase and 498,800 units/g of β-glucuronidase), β-glucuronidase (type B-1, from bovine liver), caffeic acid and methyl paraben were purchased from Sigma Chemical Co. (St. Louis, MO, USA). L(+)-Ascorbic acid was purchased from RdH Laborchemikalien GmbH & Co. KG (Seelze, Germany). Acetonitrile, methanol and ethyl acetate were LC grade and purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Milli-Q plus water (Millipore, Bedford, MA, USA) was used throughout this study. The woven gauze was purchased from T.H. Medical Gauze Inc. (Taichung, Taiwan, ROC).

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doi:10.1016/j.fct.2008.04.033

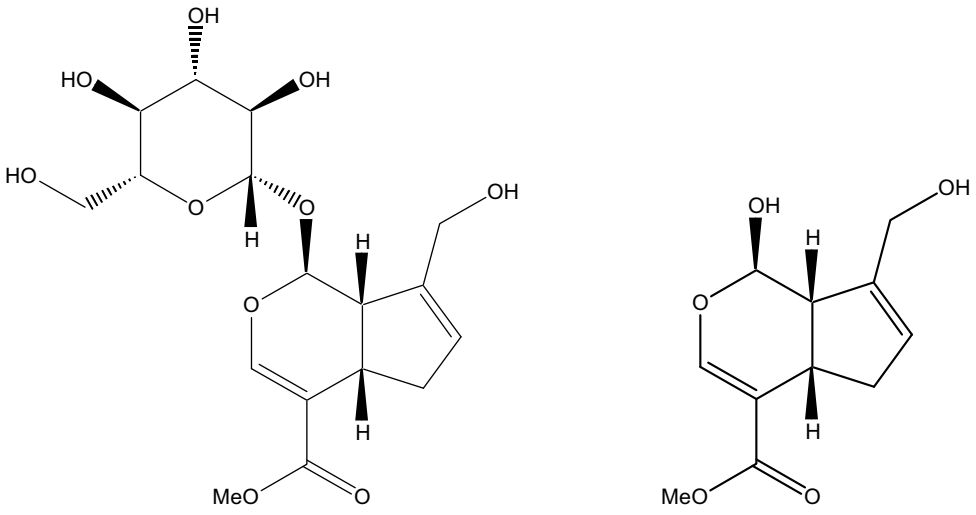

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Fig. 1. Chemical structures of geniposide (1) and genipin (2).

2.2. Instrumentation and HPLC conditions

The HPLC apparatus included a pump (LC-10AT, Shimadzu, Japan) and an UV detector (SPD-10AVP, Shimadzu, Japan) with an automatic injector (SIL-10AF, Shimadzu, Japan). The column used was Apollo C18 5 μm (250 × 4.6 mm, Alltech P.J. Cobert Associates Inc.). The detection wavelength was set at 240 nm and the flow rate was 1.0 mL/min. The elution was conducted in isocratic manner and the mobile phase consisted of acetonitrile: 0.1% phosphoric acid with 15:85 and 22:78 (v/v) for geniposide and genipin assays, respectively.

2.3. Preparation of GF decoction and quantitation of geniposide

Water (4 L) was added to 200 g crude drugs of GF and heating on a gas stove. After boiling, gentle heating was continued for about 2 h until the volume reduced to about 2 L and then the mixture was filtered with three layers of woven medical gauze while hot. The filtrate was gently boiled until the volume reduced to less than 100 mL and sufficient water was added to make 100 mL which was frozen at −20 °C for later use.

The GF decoction was diluted 1000-fold with water, of which 3 mL was added with 7 mL of methanol. After vortex and centrifugation, the supernatant (200 μL) was mixed with caffeic acid solution (200 μL, 20 μg/mL in methanol as the internal standard), and 20 μL was subject to HPLC analysis.

2.4. Animals

Male Sprague–Dawley rats, weighing 367 ± 37 g, were housed in a 12 h light–dark, constant temperature environment prior to study. A maximum of five rats were housed in each cage during the experiment. All rats were fasted 12 h before drug administration and the fasting continued for 3 h thereafter. The animal study adhered to "The Guidebook for the Care and Use of Laboratory Animals (2002)" (Published by the Chinese Society for the Animal Science, Taiwan, ROC).

2.5. Drug administration and blood collection

For intravenous dosing, genipin was dissolved in PEG 400 (50 mg/mL) and filtered through a 0.22 μm membrane. The solution was given to nine rats via tail vein at a dose of 50 mg/kg. Blood samples (0.5 mL) were withdrawn prior to dosing and at 5, 15, 30, 60, 120, 180, 240 and 360 min after dosing.

Genipin for oral dosing was dissolved in PEG 400 (10 and 20 mg/mL) and given at doses of 100 mg/kg (eight rats) and 200 mg/kg (nine rats). Blood samples (0.5 mL) were withdrawn prior to dosing and at 5, 15, 30, 60, 120, 180, 240, 360, 480, 720, 1440, 2160 and 2880 min after dosing.

GF decoction was given orally to six rats at doses of 10 and 20 g/kg. Blood samples (0.5 mL) were withdrawn prior to dosing and at 5, 15, 30, 60, 120, 180, 240, 360, 480, 720, 1440, 2160, 2880, 3600, 4320, 5760, 7200, 8640 and 10,080 min after dosing.

Drugs were given via gastric gavage and all blood samples were withdrawn via cardiac puncture. Heparin was used as anticoagulant. All blood samples were immediately centrifuged at 10,000*g* for 15 min and the plasma samples obtained were processed right away.

2.6. Determination of pKa of genipin

Genipin (22.6 mg) was dissolved in 100 mL of water and titrated with 0.1 N NaOH. The titration curve was plotted by measuring the pH values against the volumes of NaOH solution used. The mid-point of titration curve represents the p*K*a of genipin.

2.7. Quantitation of genipin and its conjugated metabolites in plasma

The concentrations of genipin in plasma were determined before and after incubations with sulfatase and β-glucuronidase (Azuma et al., 2000). Plasma (200 μL) was mixed with 100 μL of sulfatase or β-glucuronidase solution (1000 units/mL in pH 5.0 acetate buffer), 50 μL of ascorbic acid solution (100 mg/mL) and incubated at 37 °C for 30 min. After hydrolysis, plasma was partitioned with 350 μL ethyl acetate (containing 1.0 μg/mL of methylparaben as the internal standard). The ethyl acetate layer was evaporated under N_2 gas to dryness and reconstituted with an appropriate volume of mobile phase, then 20 μL was subject to HPLC analysis. For calibrator preparation, plasma was spiked with various concentrations of genipin standards, then added with pH 5.0 acetate buffer. The later procedure was the same as that described above for plasma samples. The concentrations of the standards were 0.1, 0.2, 0.3, 0.6, 1.3, 2.5, 5.0 and 10.0 μg/mL of genipin. The calibration curve was plotted by linear regression of the peak area ratios (genipin to methyl paraben) against concentrations of genipin.

2.8. Validation of genipin assay method in plasma

The precision and accuracy of the assay method was evaluated by intra-day and inter-day analysis of triplicate plasma standards over a period of 3 days. The recovery studies were conducted by spiking genipin into blank plasma in triplicates to afford 0.3, 1.3, 5.0 μg/mL, and comparing the concentration obtained from assay with the corresponding spiked concentration. LLOQ (lower limit of quantitation) represents the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy, whereas LOD (limit of detection) represents the lowest concentration of analyte in a sample that can be detected (S/N > 3).

2.9. Data analysis

For intravenous administration of genipin, the concentration of genipin sulfate was calculated by subtracting the concentration of genipin parent form from the total genipin after hydrolysis with sulfatase. The peak plasma concentrations (C_{max}) were observed from experimental data. Owing to the plasma profiles of genipin sulfate following oral administrations of genipin and GF decoction could not be satisfactorily fitted by compartment models, the areas under the plasma