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Concurrent measurement of unbound genistein in the blood, brain and bile of anesthetized rats using microdialysis and its pharmacokinetic application

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Abstract

Genistein, the major isoflavone in soybeans, has been shown to have a wide range of effects. We used an HPLC–UV combined with microdialysis method to detect unbound genistein in rat blood, brain and bile. Genistein dialysates were eluted with a mobile phase containing acetonitrile–water (40:60, v/v, pH 3.5 adjusted by 0.1% acetic acid). Samples were separated using a phenyl (5 μm) column maintained at ambient temperature. The UV detector wavelength was set at 259 nm. The flow rate was 1.0 ml/min. The limit of quantitation for genistein was 50 ng/ml. The in vitro recoveries of genistein were 31 ± 1, 13 ± 1 and 59 ± 4% in microdialysis probes of blood, brain and bile, respectively ($n = 4$). Inter- and intra-assay accuracy and precision of the analysis were less than 10% in the concentration ranges of 0.05–5.0 μg/ml. A small ratio of genistein penetrates the blood–brain barrier (BBB) and goes through hepatobiliary excretion after genistein administration (10 or 30 mg/kg, i.v.). The brain-to-blood (AUC_{brain}/AUC_{blood}) and bile-to-blood (AUC_{bile}/AUC_{blood}) distribution ratios were 0.04 ± 0.01 and 1.85 ± 0.42, respectively for the dosage of genistein 30 mg/kg. After co-administration of cyclosporine, a P-glycoprotein (P-gp) inhibitor, the distribution ratios of genistein in brain and bile were not significantly altered. These results suggest that the BBB penetration and hepatobiliary excretion of genistein may not regulated by P-gp.

Keywords: Genistein; Hepatobiliary excretion; Microdialysis; Pharmacokinetics

1. Introduction

Genistein (4',5,7-trihydroxyisoflavone; Fig. 1), one of the major isoflavones in soybeans, has been considered as potential remedy for a wide types of diseases such as osteoporosis, cardiovascular diseases and menopausal symptoms [1]. In general, soy products can lower coronary heart disease by lowering blood cholesterol levels. Following oral administration of a single soy serving for a human, genistein plasma concentration rose slowly and reached mean maximum values at about 8 h [2]. Genistein goes through phase II glucuronidation in the intestine concomitant with absorption [3].

A wide variety of analytical techniques have been applied for the quantitation of soy isoflavones in foods and biological fluids, including high-performance liquid chromatography (HPLC) with UV [4,5] and electrospray ionization mass spectrometry (LC–ESI-MS) [6], gas chromatography–mass spectrometry (GC–MS) [7,8] and capillary electrophoresis [9]. These methods require much time for sample preparation such as solid-phase extraction, liquid–liquid extraction, protein precipitation on the genistein analysis from biological samples. In contrast, the samples of microdialysate are protein-free, making it possible for direct injection onto the liquid chromatographic system with no sample clean-up required.

Genistein has been reported to interact with P-gp and inhibit P-gp-mediated drug transport [10]. However, Versantvoort et al. [11] reported that no effects of genistein were

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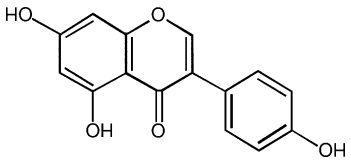

Fig. 1. Chemical structure of genistein.

observed in P-gp expressing cells. Hence, we were interested to study the interaction of genistein and cyclosporine, a P-gp inhibitor. In this study, we describe a rapid microdialysis procedure coupled with a liquid chromatographic system for the determination of unbound genistein in rat blood, brain and bile, together with its interaction with cyclosporine.

2. Experimental

2.1. Chemicals and reagents

Genistein was purchased from Sigma (St. Louis, MO, USA). Solvents and reagents of liquid chromatographic grade were obtained from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. Animals

All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of the National Research Institute of Chinese Medicine. Male specific pathogen-free Sprague–Dawley rats were obtained from the Laboratory Animal Center of the National Yang Ming University, Taipei. The animals had free access to food (Laboratory rodent diet 5P14, PMI Feeds, Richmond, IN, USA) and water until 18 h prior to being used in experiments, at which time only food was removed. Six Sprague–Dawley rats (280–320 g) were initially anesthetized with urethane 1 g/ml and α-chloralose 0.1 g/ml (1 ml/kg, i.p.), and remained anesthetized throughout the experimental period. The femoral vein was exposed for further genistein administration (10 or 30 mg/kg) and for treatment with cyclosporine 10 mg/kg 10 min before genistein administration (30 mg/kg). The body temperature of the rats was maintained at 37 °C with a heating pad.

2.3. Liquid chromatography

HPLC was performed with a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), a Rheodyne Model 7125 injector equipped with a 20 μl sampling loop and an ultraviolet detector (Linear Model 340, San Jose, CA, USA). Separation was achieved by an Alltima phenyl column (150 mm × 4 mm i.d.; particle size 5 μm). The mobile phase consisted of acetonitrile–water (40:60, v/v, pH 3.5 adjusted by 0.1% acetic acid) at flow-rate of 1 ml/min. The detection wavelength was 259 nm. Output data from the detector were integrated using an EZChrom chromatographic data system (Scientific Software, San Roman, CA, USA).

2.4. Assay validation

All calibration curves were required to have a correlation value of at least 0.999. The intra- and inter-assay variabilities were determined by quantitating six replicates at concentrations of 0.01, 0.05, 0.1, 0.5, 1 and 5 μg/ml using the HPLC method described above on the same day and six consecutive days, respectively. The accuracy (% bias) was calculated from the mean value of observed concentration (C_{obs}) and the nominal concentration (C_{nom}) as follows: accuracy: (% bias) = $[(C_{obs} - C_{nom})/C_{nom}] \times 100$. The relative standard deviation (R.S.D.) was calculated from the observed concentrations as follows: precision (% R.S.D.) = [standard deviation (S.D.)/C_{obs}] × 100.

2.5. Microdialysis experiment

Blood, brain and bile microdialysis systems consisted of a microinjection pump (CMA/100, Stockholm, Sweden), microdialysis probes and stereotaxic frame. The dialysis probes for blood (10 mm in length), brain (3 mm in length) and bile (7 cm in length) were made of silica capillary in a concentric design with the tips covered by dialysis membrane (Spectrum, 150 μm outer diameter with a cut-off at the nominal molecular mass of 18,000, Laguna Hills, CA, USA). The blood microdialysis probe was positioned within the jugular vein toward the right atrium and then perfused with anticoagulant citrate dextrose, ACD solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM) at a flow-rate of 2.5 μl/min.

The bile duct microdialysis probe was constructed in our laboratory, based on the design originally described by Scott and Lunte [14]. A 7 cm dialysis membrane was inserted into polyethylene tubing (PE-60; 0.76 mm i.d. × 1.22 mm o.d., Clay-Adams, NJ, USA). The ends of the dialysis membrane and PE-60 were inserted into silica tubing (40 mm i.d × 140 mm o.d., SGE, Australia) and PE-10 (0.28 mm i.d. × 0.61 mm o.d.), respectively. Both ends of the tubing and the union were cemented with epoxy and the epoxy was allowed to dry at least for 24 h. For post bile duct cannulation, the microdialysis probe was then perfused with Ringer's solution (147 mM Na^+; 2.2 mM Ca^{2+}; 4 mM K^+; pH 7.0) at 2.5 μl/min flow rate.

After the implantation of blood and bile microdialysis probes, the rat was immobilized in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The skull was surgically exposed, and a hole was trephined into the skull based on stereotaxic coordinates. The brain microdialysis probe was placed into the right striatum (0.2 mm anterior to bregma, 3.0 mm lateral to midline and 7.5 mm lower to tip). The brain microdialysis probe was perfused with Ringer's solution at the flow-rate of 2.5 μl/min.