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Identification of absolute conversion to geraldol from fisetin and pharmacokinetics in mouse

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ABSTRACT

Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a flavonoid found in several fruits, vegetables, nuts, and wine and has anti-oxidant, anti-inflammatory, and anti-angiogenic properties. Geraldol is the 3′-methoxylated metabolite of fisetin (3,4′,7-trihydroxy-3′-methoxyflavone). The concentration of fisetin and geraldol in mouse plasma was determined by LC–MS/MS, following direct protein precipitation. These concentrations were determined after administration of fisetin at doses of 2 mg/kg (*i.v.*) and 100 and 200 mg/kg (*p.o.*). The method was validated in terms of linearity, accuracy, precision, matrix effect, and stability. The pharmacokinetics parameters of fisetin and geraldol were successfully determined using a validated method in mice. Results indicated that fisetin was very rapidly methylated to geraldol *in vivo*. Following administration of fisetin, it was observed that the C_{max} and AUC values for geraldol were higher than those of fisetin. The absolute bioavailability of fisetin was calculated as 7.8% and 31.7% after oral administration of 100 and 200 mg/kg fisetin, respectively. This method was successfully applied to determine the pharmacokinetic parameters of fisetin and its main metabolite geraldol in mouse plasma. Geraldol was the dominant circulating metabolite after fisetin administration *in vivo*.

1. Introduction

Fisetin (3,3′,4′,7-tetrahydroxyflavone; Fig. 1A) is a member of the flavonoid class of compounds, and is found in fruits (apples, strawberries, persimmons, grapes), vegetables (onions, cucumbers), and derived products (wine) [1,2]. Flavonoids have attracted significant scientific consideration owing to their biological functions. For example, fisetin possesses anti-oxidant, anti-inflammatory, anti-angiogenic, anti-septic, and cytoprotective activities [3–9]. In addition, fisetin showed anti-fungal effect against *Cryptcococcus neoformans* following the biosynthesis of ergosterol [10]. Geraldol (3,4′,7-trihydroxy-3′-methoxyflavone; Fig. 1B) is a 3′-methoxylated metabolite of fisetin, and is known to be a widely generated and active metabolite *in vivo* [11]. Fisetin is methylated in the human liver to geraldol by methyltransferases, with a K_m value of 8.6 μM [12]. The methyltransferase involved was probably catechol-*O*-methyl transferase (COMT) as fisetin possesses a catechol ring [11]. This methoxylated metabolite was found to be more cytotoxic than its parent compound, and could also inhibit endothelial cell migration and proliferation [11]. Geraldol showed anti-caspase-3-dependent mitotic checkpoint inactivation to the outcome of anti-mitotic cancer therapy [13,14].

Although fisetin and geraldol have demonstrated various pharmacological activities, pharmacokinetic information on these two compounds has been limited. In a previous study, fisetin, after intraperitoneal (*i.p.*) administration (223 mg/kg) was found to reach a concentration of 2.5 μg/mL at 15 min in the plasma, and displayed a relatively long terminal half-life of 3.12 h [11]. While the pharmacokinetic parameters of sulfate and glucuronide fisetin conjugates were determined after intravenous (*i.v.*) and oral administration of fisetin in rats [15], there is no data about the pharmacokinetic parameters of fisetin. Moreover, the pharmacokinetics of fisetin-derived geraldol in the plasma has not been shown.

Here, we successfully developed a selective and sensitive liquid chromatography-tandem mass spectrometry (LC–MS/MS) method to quantify the concentrations of fisetin and geraldol in mouse plasma after oral and *i.v.* administration of fisetin. We also reported the pharmacokinetic parameters of fisetin and geraldol in mice.

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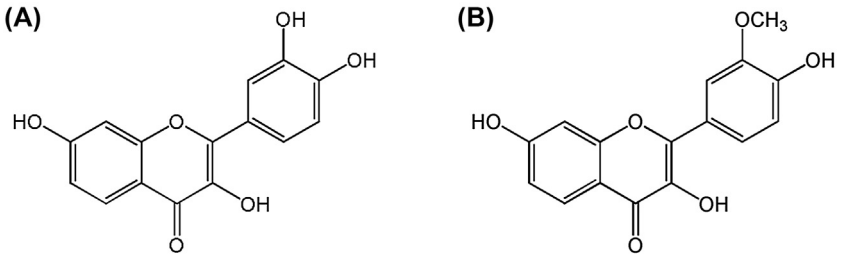

Fig. 1. Chemical structures of fisetin (A) and geraldol (B).

2. Experimental

2.1. Chemicals and reagents

Fisetin, terfenadine (internal standard, IS), polyethylene glycol 300 (PEG_{300}), and formic acid (FA) were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Geraldol was purchased from Extrasynthese (Genay Cedex, France). MS-grade water and acetonitrile (ACN) were purchased from Fisher Scientific Korea (Seoul, Korea).

2.2. Plasma sample preparation

Male 5-week-old ICR mice were provided by Orient Co. (Seongnam, Korea) for the study of fisetin pharmacokinetics. Upon arrival, mice were randomly housed, with 4 to 6 per cage, in a controlled environment (Relative humidity: 60%, temperature: maintained at 25 °C) under a 12 h:12 h light/dark cycle. All animal procedures complied with the guidelines issued by the Society of Toxicology (USA; 1989) and this study was approved by the Institutional Review Board of Kyungpook National University (2014-160).

After fasting for 12 h before experimentation, the mice (mean body weight 31 ± 2 g) were randomly divided into three groups: one group (n = 3) received an *i.v.* administration of fisetin (2 mg/kg), another group (n = 3) received an oral administration of fisetin (100 mg/kg), and the last group (n = 3) received an oral administration of fisetin (200 mg/kg). Fisetin was dissolved in 60% PEG_{300}. Approximately 20–40 μL of blood samples were taken from the tail vein after *i.v.* fisetin administration at 2, 5, 10, 15, 30, 60, 120, and 240 min, and collected in heparinized tubes. Similarly, blood samples were collected at 0, 5, 10, 15, 30, 60, 120, and 240 min after oral fisetin administration. The samples were obtained by centrifuging blood samples at 4000 *g* for 15 min at 4 °C and isolating the supernatants, which were stored at −80 °C until analysis.

Plasma samples (10 μL) were mixed with 90 μL ACN in 0.1% formic acid containing 5 μM IS solution. This solution was then vortexed for 1 min and centrifuged at 13,000 rpm for 10 min at 4 °C, with 90 μL of the resulting supernatant transferred to autosampler vials and injected into the LC–MS/MS system.

2.3. LC–MS/MS

The LC system consisted of a G1312A bin bump (Cohesive Technologies, USA), G1322A degasser, 1100 series COLCOM (Agilent, USA), and 1100 series auto-sampler. An ACE® 5C18 column (5 μm, 50 mm × 2.1 mm, Advanced Chromatography Technologies, Scotland) and a guard C18 column (2 mm, 2.1 mm i.d., Phenomenex, USA) were used for LC separation. A gradient program was employed with the mobile phase combining solvent A (0.1% formic acid in ACN) and solvent B (0.1% formic acid in water) at a flow rate of 250 μL/min. The gradient was as follows: 10–10% A (0–0.5 min), 10–95% A (0.5–1.5 min), 95–95% A (1.5–2.5 min), 95–5% A (2.5–2.7 min), 5–5% A (2.7–5.0 min). During the analysis, the column oven was maintained at 40 °C.

Samples were analyzed using an LC system coupled to an API 3000 triple quadrupole mass spectrometer (AB SCIEX, USA) with an electrospray ionization (ESI) source. The mass spectrometer was operated in the positive ESI mode with nitrogen as the curtain gas, nebulizer gas, and collision gas, with optimum values set at 10, 8, and 6 psi respectively. The ESI needle voltage was adjusted to 5500 V and the turbo-gas temperature was set at 375 °C. For all the low-energy collision dissociation using the multiple reaction monitoring scan, the transitions recorded were form *m*/*z* precursor protonated molecule → *m*/*z* product ion, as shown: *m*/*z* 287.1 → 137.1 for fisetin, *m*/*z* 301.2 → 286.2 for geraldol, and *m*/*z* 472.1 → 436.3 for the IS, at a collision energy of 45 eV for fisetin, 35 eV for geraldol, and 36 eV for the IS (Fig. 2). Data procurement was controlled by Analyst 1.5 software.

2.4. Method validation

2.4.1. Linearity and calibration curve

Stock solutions of fisetin and geraldol were prepared in dimethyl sulfoxide (DMSO) at a concentration of 30 mg/mL. Ten microliters of each stock solution was added to 90 μL of drug-free mouse plasma. The final concentrations of fisetin and geraldol in the calibration standards were 0.02, 0.05, 0.1, 0.2, 0.5, 1, 5, 10, and 15 μg/mL. Aliquots (10 μL) of standard plasma samples were then processed as described above. Calibration curves were constructed by plotting the peak-area ratios of analyte or the IS versus the concentrations of fisetin and geraldol in mouse plasma.

2.4.2. Accuracy and precision

The precision and accuracy of the methodology were evaluated by analyzing seven replicates of mouse plasma spiked with known concentrations of fisetin (0.02, 0.5, 10, and 15 μg/mL) or geraldol (0.02, 0.1, 5, and 15 μg/mL). The intra-day accuracy and precision of fisetin and geraldol were determined by repeating the assay on the same day at each concentration. The inter-day accuracy and precision were evaluated on five consecutive days at each concentration.

2.4.3. Stability

To check freeze-thaw stability, all stability as the mean of percentage remains, was determined through three freeze (−20 °C)/thaw (room temperature) cycles. The ion suppression by matrix was tested by comparing the ion intensity of fisetin of the post-extraction spiked samples to those of directly evaporated standard solvent.

2.5. Pharmacokinetic study of fisetin and geraldol

A non-compartmental model was used to evaluate the pharmacokinetic parameters of fisetin and geraldol using WinNonlin software (Version 2.1, Scientific Consulting, KY, USA). The parame-