Drug Metabolism and Pharmacokinetics xxx (2018) 1–7

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Regular Article

Selective inhibition of CYP2C8 by fisetin and its methylated metabolite, geraldol, in human liver microsomes

Riya Shrestha [a, 1], Ju-Hyun Kim [b, 1], Wongshik Nam [a], Hye Suk Lee [b], Jae-Mok Lee [c, **], Sangkyu Lee [a, *]

[a] BK21 Plus KNU Multi-Omics-based Creative Drug Research Team, College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu, 41566, Republic of Korea
[b] BK21 PLUS Team for Creative Leader Program for Pharmacomics-based Future Pharmacy and Integrated Research Institute of Pharmaceutical Sciences, College of Pharmacy, The Catholic University of Korea, Bucheon, 14662, Republic of Korea
[c] Department of Periodontology, School of Dentistry, Kyungpook National University, Daegu, 41940, Republic of Korea

ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form
20 September 2017
Accepted 12 December 2017
Available online xxx

Keywords:
Fisetin
Geraldol
Cytochrome P450
Inhibition
Human liver microsomes
Interaction

ABSTRACT

Fisetin is a flavonol compound commonly found in edible vegetables and fruits. It has anti-tumor, antioxidant, and anti-inflammatory effects. Geraldol, the *O*-methyl metabolite of fisetin in mice, is reported to suppress endothelial cell migration and proliferation. Although the *in vivo* and *in vitro* effects of fisetin and its metabolites are frequently reported, studies on herb–drug interactions have not yet been performed. This study was designed to investigate the inhibitory effect of fisetin and geraldol on eight isoforms of human cytochrome P450 (CYP) by using cocktail assay and LC-MS/MS analysis. The selective inhibition of CYP2C8-catalyzed paclitaxel hydroxylation by fisetin and geraldol were confirmed in pooled human liver microsomes (HLMs). In addition, an IC_{50} shift assay under different pre-incubation conditions confirmed that fisetin and geraldol shows a reversible concentration-dependent, but not mechanism-based, inhibition of CYP2C8. Moreover, Michaelis-Menten, Lineweaver-burk plots, Dixon and Eadie-Hofstee showed a non-competitive inhibition mode with an equilibrium dissociation constant of 4.1 μM for fisetin and 11.5 μM for geraldol, determined from secondary plot of the Lineweaver-Burk plot. In conclusion, our results indicate that fisetin showed selective reversible and non-competitive inhibition of CYP2C8 more than its main metabolite, geraldol, in HLMs.

1. Introduction

Fisetin (Fig. 1) is a naturally occurring flavonol compound, which is abundant in vegetables and fruits that people commonly consume in everyday life, such as strawberries, apples, and onions [1]. In addition, fisetin is commonly found in trees and shrubs of the Fabaceae family and is traditionally used as a yellow dye and in wine [2]. The pharmacological effects of fisetin have extensively been reported in *in vitro* and *in vivo* studies. Fisetin has anti-tumor, antioxidant, and anti-inflammatory activities [3]. It has been shown to delay tumor growth when administered alone, and to allow a reduction in cisplatin dose when administered as a combination therapy that was reported to reduce the side effects of cisplatin. This verified that fisetin exhibits effective antitumor activity [4]. Geraldol (Fig. 1) is a methoxylated metabolite of fisetin in mice [5,6] and has recently been isolated from *Akschindlium godefroyanum* [7]. Geraldol is reported to have a greater cytotoxic effect than fisetin. In addition, it inhibits endothelial cell migration and proliferation, as does fisetin [6].

Cytochrome P450 (CYP) is an oxidizing enzyme that is generally involved in the terminal stage of the electron transport chain [8]. In order to prevent adverse reactions, a drug–drug interaction study to evaluate CYP inhibition by specific drug combinations is required. CYP1A2 is one of the major CYP enzymes in human liver (~13%) and is constitutively expressed in the livers of rodents and

* Corresponding author. College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, 80 Daehak-ro, Buk-gu, 41566, Republic of Korea.
** Corresponding author. Department of Periodontology, School of Dentistry, Kyungpook National University, 2177 Dalgubeol-daero, Jung-gu, Daegu, 41940, Republic of Korea.
E-mail addresses: leejm@knu.ac.kr (J.-M. Lee), sangkyu@knu.ac.kr (S. Lee).
[1] These authors contributed equally.

https://doi.org/10.1016/j.dmpk.2017.12.006
1347-4367/

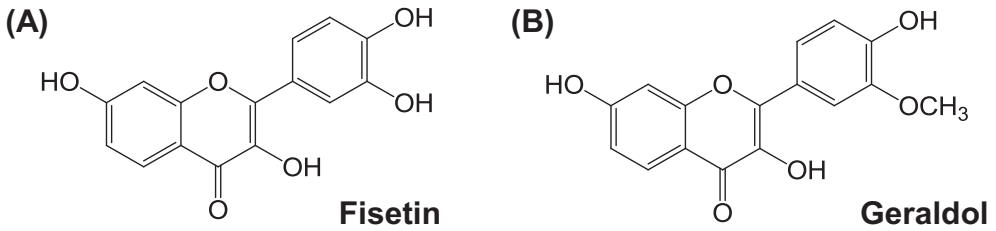

Fig. 1. Chemical structures of (A) fisetin and (B) geraldol.

humans [9]. Although CYP2B6 is regarded as a minor CYP in the human liver, it can metabolize approximately 10% of clinically useful drugs and a number of procarcinogens and environmental chemicals [10]. CYP2D6 metabolizes approximately 25% of marketed drugs and mediates drug–drug interactions through the poor metabolizer phenotype [11]. CYP3A4 is the most abundant CYP in human liver, metabolizing approximately 50% of marketed drugs [12].

The CYP2C subfamily is one of the major CYPs, accounting for approximately 25–30% of all CYP present in human liver, and which comprises four isoforms: CYP2C8, 2C9, 2C18, and 2C19 [13]. Of the CYP2C subfamily, CYP2C8 accounts for approximately 7% of total hepatic CYP content, and is involved in the metabolism of more than 50 drugs in clinical use, such as paclitaxel, repaglinide, and rosiglitazone [14,15]. In particular, anticancer drugs such as paclitaxel, imatinib, and enzalutamide are substrates of CYP2C8 [16]. In patients receiving drugs that affect CYP2C8 activity in combination with anticancer drugs, careful attention should be paid to potential interactions. Many studies have reported the pharmacological effects of fisetin and its metabolite, geraldol. However, to date, little information about this potential herb–drug interaction has been available. Therefore, we investigated the potential selective inhibitory effect of fisetin and geraldol on CYP2C8 to predict herb–drug interactions using cocktail assay and an LC-MS/MS system in pooled HLMs.

2. Materials and methods

2.1. Reagents and chemicals

Fisetin, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were purchased from Sigma–Aldrich Corporation (St. Louis, MO). Geraldol was purchased from Extrasynthese (Geney Cedex, France). Pooled HLMs (BD UltraPool^{TM} HLM 150$^{®}$, 400 pmol/mg) were purchased from Corning Gentest (Woburn, MA). Reduced β-nicotinamide adenine dinucleotide phosphate (β-NADPH) was obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). All chemicals used in the study were of analytical grade.

2.2. Enzyme assay in pooled human liver microsomes

We determined the inhibitory effects of fisetin and geraldol on the activity of seven CYP isoforms in human liver microsomes. The inhibitory effects were examined by assessing the metabolic reactions of seven different CYP-specific substrates that are listed as follows: CYP1A2-mediated phenacetin (80 μM) metabolism, CYP2A6-catalyzed coumarin (4 μM) metabolism, CYP2B6-catalyzed bupropion (20 μM) metabolism, CYP2C8-mediated paclitaxel hydroxylation (10 μM), CYP2C9-catalyzed diclofenac 4-hydroxylation (10 μM), CYP2C19-mediated omeprazole 5-hydroxylation (20 μM), CYP2D6-catalyzed dextromethorphan (10 μM) metabolism, and CYP3A4-mediated midazolam (5 μM) metabolism [17]. Pooled human liver microsomes (0.5 mg/mL) were incubated with 0.1 M potassium phosphate buffer (pH 7.4) and made to a final incubation volume of 0.1 mL. The incubation medium comprised a β-NADPH-generating system (NGS), which consisted of 0.1 M glucose 6-phosphate, 10 mg/mL β-NADPH, 1.0 U/mL glucose-6-phosphate dehydrogenase, and probe substrates. The reaction was induced for 30 min after the addition of NGS, after which 100 μL of acetonitrile containing 0.1% formic acid and 2.5 μL of the internal standard solution (50 μM reserpine) in methanol were added to end the reaction. Incubations were carried out in duplicate. After centrifugation at 13,000 × *g* for 15 min, 10 μL of the supernatant was injected into a C18 column for LC-MS/MS analysis.

2.3. Investigation of the mechanism of inhibition of CYP2C8 by fisetin

To determine the mechanism of inhibition of CYP2C8 by fisetin and geraldol, the possibility of mechanism-based inhibition was tested. Each reaction mixture, consisting of fisetin or geraldol and pooled HLMs (0.5 mg/mL), was preincubated for 20 min with or without β-NADPH (1 mM). After pre-incubation, they were incubated with paclitaxel and NGS for 60 min. The reaction was stopped by the addition of 100 μL of acetonitrile containing 0.1% formic acid and 2.5 μL of the internal standard solution (50 μM reserpine) in methanol. After centrifugation at 13,000 × *g* for 10 min, 10 μL of the supernatant was analyzed using a C18 column *via* LC-MS/MS. To characterize the mechanism of inhibition by fisetin and geraldol on CYP2C8 activity, Michaelis-Menten saturation curve and Lineweaver-Burk, Dixon, and Eadie-Hofstee plots were constructed following incubation with fisetin or geraldol at 0–25 μM and paclitaxel at 2.5–25 μM in 0.1 M potassium phosphate buffer (pH 7.4) for 60 min at 37 °C.

2.4. Instruments and equipment

All measurements were performed using LC-MS/MS in a multiple-reaction monitoring mode (MRM) as described in a previous report [17]. The LC-MS/MS system utilized the API3000 Triple Quadrupole mass spectrometer (AB Sciex, Framingham, USA), which was coupled with the Agilent 1100 LC system. Electrospray ionization was performed in the positive mode at a spray voltage of 3500 V. Nitrogen was used as a sheath and auxiliary gas at optimum values of 45 and 20 (arbitrary units). The vaporizer and capillary temperatures were set at 150 °C and 300 °C, respectively. An Inertsil$^{®}$ ODS-2 (3 μm, 2.1 × 150 mm) column (GL Sciences Inc., Torrance, CA) was used for LC analysis. The mobile phase consisted of acetonitrile (mobile phase A) that contained 0.1% formic acid, and water (mobile phase B) that contained 0.1% formic acid. The mobile phase was introduced at a 30° angle at a flow rate of 0.22 mL/min.

Please cite this article in press as: Shrestha R, et al., Selective inhibition of CYP2C8 by fisetin and its methylated metabolite, geraldol, in human liver microsomes, Drug Metabolism and Pharmacokinetics (2018), https://doi.org/10.1016/j.dmpk.2017.12.006