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Rutaecarpine effects on expression of hepatic phase-1, phase-2 metabolism and transporter genes as a basis of herb–drug interactions

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ABSTRACT

Ethnopharmacological relevance: Rutaecarpine is an alkaloid of *Evodia rutaecarpa* which is traditionally used to treat human diseases. Rutaecarpine has been used in combination with other drugs in the treatment of disorders and found to produce herb–drug interactions. The basis of these herb–drug interactions is not completely understood.

Aim of study: To examine the effects of rutaecarpine on the expression of drug processing genes, including Phase-1 (P450 enzyme genes), Phase-2 (glucuronidation and sulfation genes) and Phase-3 (drug transporters) in liver of mice.

Materials and methods: Mice were orally administered rutaecarpine at the doses of 10, 20, and 30 mg/kg for consecutive 7 days. Twenty-four hours after the last dose, blood and liver were collected. Total RNA was isolated, purified, and subjected to real-time RT-PCR analysis of genes of interest.

Results: Rutaecarpine administration induced Cyp1a2, 2b10 and 2e1 as previously reported. Cyp3a11 and Cyp4a10 were also induced. For phase-2 enzyme genes, rutaecarpine increased glucuronyltransferases (Ugt1a1 and Ugt1a6), but had no effects on sulfotransferase (Sult1a1 and Sult1b1). Most interestingly, rutaecarpine increased hepatic uptake of organic anion transporting peptides (Oatp1a1, Oayp1a4, Oatp1b2, and Oatp2b1) and induced efflux transporter such as multidrug resistance-associated proteins (Mrp1, Mrp2, Mrp3, and Mrp4), especially at the doses of 20 mg/kg and above.

Conclusion: The interactions of rutaecarpine with drugs involve not only the induction of cytochrome P450 enzyme genes, but also the induction of hepatic transporters and phase-2 enzyme genes. The effects of rutaecarpine on these drug processing genes could play integrated roles in producing herb–drug interactions.

1. Introduction

Rutaecarpine (8,13-dihydro-7H-indolo-[2′,3′:3,4]-pyrido-[2,1-b]-quinazolin-5-one) is one of the main active alkaloids of *Evodia rutaecarpa* (Wu-Chu-Yu) with a variety of biological activities (Lee et al., 2008; Jia and Hu, 2010; Chen et al., 2012). Wu-Chu-Yu is a well-known herbal drug used for hypertension. Rutaecarpine and evodiamine are main bioactive components of the medicine (Xu et al., 2012) and high purity of *Evodia rutaecarpa* extract is preferred for topical preparation to increase drug delivery (Chen et al., 2012). The beneficial cardiovascular effects of rutaecarpine include inotropic and chronotropic, and vasorelaxant effects (Jia and Hu, 2010). Our Laboratory has found that Rutaecarpine is effective against AngII-induced vascular smooth muscle cell proliferation, and protects against abdominal aortic constriction (ACC)-induced cardiotrophy (Wu et al., 2010). Rutaecarpine has been used traditionally in China for treatment of headache, abdominal pain, postpartum hemorrhage, dysentery and amenorrhea, and recently for anti-inflammation, anti-thrombotic, and anti-obesity (Lee et al., 2008; Jia and Hu, 2010).

Since rutaecarpine has many beneficial effects, it has been used in combination with traditional Chinese medicines, or in combination with Western medicines in the treatment of various human diseases not only in China (Jia and Hu, 2010), but also in other Asian countries (Lee et al., 2008). The combined use of herbs and drugs has increased the possibility of pharmacokinetic and pharmacodynamic interactions, which could enhance or attenuate the drug efficacy and toxicity (Guengerich, 1997; Liu et al., 2011; Choi et al., 2011; Xu et al., 2012). Herb–drug interaction is an emerging

Abbreviations: Cyp, cytochrome P450; Ugt, glucuronylotransferase; Sult, sulfotransferase; Oatp, organic anion transporting peptide; Mrp, multidrug resistance-associated protein; Rut, rutaecarpine

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issue in traditional Chinese medicine research (Liu et al., 2011; Mukherjee et al., 2011; Wu et al., 2012), especially during the concomitant administration of herbal extracts and prescription/over-the-counter drugs (Mukherjee et al., 2011). Rutaecarpine is such an example. Rutaecarpine has been shown to increase the metabolism and elimination of caffeine (Tsai et al., 2005; Noh et al., 2011), theophylline (Ueng et al., 2005; Jan et al., 2005), acetaminophen (Lee et al., 2007), and *Rhizoma coptidis* alkaloids (Ma et al., 2011), indicating that rutaecarpine could act on drug processing
genes in the liver, which would include Phase-1 (cytochrome P450), Phase-2 (conjugation reaction), and Phase-3 metabolism (drug transporters).

Rutaecarpine treatment is well-known to induce the activity of hepatic cytochrome P450 enzyme/genes in animals, particularly CYP1A1 and 1A2 enzyme genes (Ueng et al., 2001, 2002a, 2002b; Lee et al., 2004; Han et al., 2009), at both protein and activity levels. In addition, CYP2B1, 2E1 and 3A enzyme activities are also induced by rutaecarpine *in vivo* (Ueng et al., 2002a; 2002b). Curiously, in liver microsome incubations, rutaecarpine is an inhibitor of CYP1A enzymes when added in vitro (Ueng et al., 2002c). Thus, induction of CYP enzyme has been proposed as a mechanism of rutaecarpine effects on increased metabolism of theophylline (Ueng et al., 2005), acetaminophen (Lee et al., 2007), and caffeine (Noh et al., 2011).

Glucuronidation, glutathione conjugation and sulfation conjugation represent the three most prevalent classes of phase-2 metabolism, which may occur directly on the parent compounds that contain appropriate structural motifs, or, as is usually the case, on functional groups added or exposed by phase-1 oxidation (Zamek-Gliszczynski et al., 2006). Phase-2 conjugations are an important aspect of herb–drug interactions (Li et al., 2012). The Phase-2 conjugation reactions are important for acetaminophen, caffeine, and theophylline excretion through the bile (Lee et al., 2004; Tsai et al., 2005; Ueng et al., 2005; Noh et al., 2011). Effects of rutaecarpine on cytochrome P450s have been extensively studied; however, its effects on Phase-2 conjugation metabolism have not been adequately investigated. While some studies suggest that rutaecarpine does not affect glucuronidation and glutathione conjugation (Ueng et al., 2002b, 2002c), a recent study indicated that Wu-Chu-Yu pretreatment decreased the systemic exposure of the *Rhizoma coptidis* alkaloids by inducing hepatic UGT1A1 (Ma et al., 2011).

Transporters influence the disposition of chemicals within the body by participating in absorption, distribution, and elimination (Klaassen and Aleksunes, 2010), and is one of important mechanisms for herb–drug interactions (Choi et al., 2011; Li et al., 2012). However, nothing is known about effects of rutaecarpine on liver drug transporters. The present study was therefore designed to examine the effects of rutaecarpine on hepatic uptake organic anion transporting peptides (Oatp1a1, Oatp1a4, Oatp1b2 and Oatp2b1) and efflux transporters of multidrug resistance-associated protein genes (Mrp1, Mrp2, Mrp3, and Mrp4). In addition, the effects of rutaecarpine on major phase-1 CYP enzyme genes and major Phase-2 conjugation enzyme genes were also examined.

2. Materials and methods

2.1. Reagent and Animals

Rutaecarpine (Rut, purity $>98\%$ via HPLC) was provided by National Institute for Pharmaceutical and Biological Products (Beijing, China). All other chemicals were regent grade.

Eight-week-old male Kunming (KM) mice, weighing 18–22 g, were obtained from the Experimental Animal Center of Third Military Medical College (Chongqing, China; Certificate no. SCXK 2007-0005). Mice were kept in a SPF-grade animal facilities (Certificate no. SYXK 2011-004) at Zunyi Medical College, with regulated environment ($22\pm1\,^{\circ}C$, $50\pm2\%$ humidity and a 12 h:12 h light:dark cycle) and free access to water and laboratory chow. All animal procedures follow the WHO Guidance of Humane Care and Use of Laboratory Animals.

2.2. Experimental design

All animals were adapted for one week, and randomly divided into four groups: solvent control (corn oil), Low (10 mg/kg), middle (20 mg/kg), and high dose (30 mg/kg) of rutaecarpine, at the volume of 10 ml/kg intragastric gavage (i.g.) daily, for consecutive seven days. The dose selection was based on the literature and our prior studies (Wu et al., 2010). Twenty-four hours after dosing, blood and livers were collected. Approximately 50–100 mg liver was immediately put into 1.0 ml TRIzol solution for RNA isolation.

2.3. Animal general health

Mice were monitored during the 7-day dosing period, including the body weight and animal activities. At the end of experiment, blood was collected to separate serum. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed as biomarkers of liver injury with automatic blood analyzer in Affiliated Hospital of Zunyi Medical College. Liver weights were recorded and a portion of the liver was fixed in 10% neutral formalin, processed by standard histological techniques, stained with hematoxylin and eosin, and examined for morphological evidence of liver injury.

2.4. RNA isolation and real-time RT-PCR analysis

Approximately 50–100 mg of liver was homogenized in 1 ml Trizol (TakaRa Biotechnology, Dalian, China). Total RNA was extracted according to the manufacturer′s instructions, followed by purification with Total RNA (Mini) Kit (Watson Biotechnology, Shanghai, China). The quality and quantity of RNA was determined by the 260/280 ratios Purified RNA was reversed transcribed with the High Capacity Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA). The primers were designed with the Primer3

Table 1
Primer sequences.

Gene	Access	Forward	Reverse
Cyp1a2	NM_009993	GACATGGCCTAACGTGCAG	GGTCAGAAAGCCGTGGTTG
Cyp2b10	AF128849	AAGGAGAAGTCCAACCAGCA	CTCTGCAACATGGGGGTACT
Cyp2e1	NM_021282	TCCCTAAGTATCCTCCGTGA	GTAATCGAAGCGTTTGTTGA
Cyp3a11	NM_007818	ACAAACAAGCAGGGATGGAC	CCCATATCGGTAGAGGAGCA
Cyp4a10	NM_010011	CACACCCTGATCACCAACAG	TCCTTGATGCACATTGTGGT
G3PDH	M32599	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT
Mrp1	NM_008576	GCCCTCTTTGCAGTCATCTC	CAGTCTCTCCACTGCCACAA
Mrp2	NM_013806	TCCTAGACAGCGGCAAGATT	GCTAGAGCTCCGTGTGGTTC
Mrp3	NM_029600	TGGTCATGCTGTCAGCTTTC	AAGGACTGAGGGGAACGAAT
Mrp4	BC150822	GCAAAGCCCATGTACCATCT	ACCACGGCTAACAACTCACC
Oatp1a1	NM_013797	ATCCAGTGTGTGGGGACAAT	GCAGCTGCAATTTTGAAACA
Oatp1a4	NM_030687	GGAAGATTGGACACGCATCT	GGCATTGTGACTGAAGCAGA
Oatp1b2	NM_020495	CAAACTCAGCATCCAAGCAA	GGCTGCCAAAAATATCCTGA
Oatp2b1	NM_020495	CTAGGCCAAATGCCAGAAAG	TTGCTTGGATGCTGAGTTTG
Sult1a1	NM_133670	GGATGTAGCTGAGGCAGAGG	CAGCTCCCAGTGGCATTTAT
Sult1b1	NM_019878	GGTGGGAAAAGAGGGAAGAG	AAGGCCTCTTCATCCAAGGT
Ugt1a1	NM_201645	ACACCGGAACTAGACCATCG	ATACCATGGGAGCCAGAGTG
Ugt1a6	NM_145079	ATACCATGGGAGCCAGAGTG	ACCAGAACTGTGAGGGTTGG