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Effects of diosmetin on nine cytochrome P450 isoforms, UGTs and three drug transporters *in vitro*

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ABSTRACT

Diosmetin (3′, 5, 7-trihydroxy-4′-methoxyflavone), a natural flavonoid from traditional Chinese herbs, has been used in various medicinal products because of its anticancer, antimicrobial, antioxidant, estrogenic and anti-inflammatory activity. However, flavonoids could affect the metabolic enzymes and cause drug-drug interactions (DDI), reducing the efficacy of co-administered drugs and potentially resulting in serious adverse reactions. To evaluate its potential to interact with co-administered drugs, the IC_{50} value of phase I cytochrome P450 enzymes (CYPs), phase II UDP-glucuronyltransferases (UGTs) and hepatic uptake transporters (organic cation transporters (OCTs), organic anion transporter polypeptides (OATPs) and Na^+-taurocholate cotransporting polypeptides (NTCPs)) were examined *in vitro* by LC-MS/MS. Diosmetin showed strong inhibition of CYP1A2 in a concentration-dependent manner. The intensity of the inhibitory effect was followed by CYP2C8, CYP2C9, CYP2C19 and CYP2E1. For CYP2A6, CYP2B6, CYP2D6 and CYP3A4, diosmetin was found to have no significant inhibitory effects, and the induction effect on CYPs was not significant. For UGTs, diosmetin had a minimal inhibitory effect. In addition, the inhibitory effects of diosmetin on OATP and OCT1 were weak, and it had little effect on NTCP. This finding indicated that drug-drug interactions induced by diosmetin may occur through co-administration of drugs metabolized by CYP1A2.

1. Introduction

Biotransformation, the metabolism of endogenous and exogenous xenobiotics in the body catalyzed through a variety of metabolic enzymes, is the main mechanism by which the body maintains homeostasis. The liver is the main organ of biotransformation through related enzymes, such as cytochrome P450 enzymes (CYPs), UDP-glucuronyltransferase (UGTs), hepatic transporter and others, which are presented in liver microsomes, cytosol and mitochondria. The inhibition of these biotransformation-related proteins was the most common mechanism underlying drug-drug interactions (DDI) when patients were on multiple drug therapy. This is one of the major causes of drug withdrawal from the market.

It is generally accepted that phase I drug-metabolizing enzymes, CYPs, metabolize nearly 90% of pharmaceutical agents. CYP superfamilies include CYP1, CYP2 and CYP3. CYP3A4 is most abundantly expressed in the liver and accounts for approximately 34% of drug metabolism, which is followed by CYP2D6 at 19%, CYP2C8 and CYP2C9 at 16%, and CYP2C19 and CYP1A2 at 8% (Lewis, 2003; Zanger et al., 2008). Inhibition or induction of any isoforms of CYPs has been recognized as the pivotal cause of metabolic DDI. The metabolites formed by Phase I drug-metabolizing enzymes are further metabolized by phase II drug-metabolizing enzymes, such as UGTs, sulfotransferases, and glutathione S-transferases. Among these enzymes, UGTs contribute the most to drug metabolism (Jancova et al., 2010). Evidence has shown that UGTs participate in 35% of the drug metabolism related to phase II enzymes. Increasing studies have demonstrated that the inhibition of UGTs may mediate DDI and cause serious adverse reactions. Chemical medicine, herb medicine and the active ingredients could also inhibit the activity of UGTs (Kim et al., 2016; Li

Abbreviations: CYPs, Cytochrome P450 enzymes; DDI, drug-drug interactions; HDI, herb-drug interactions; HLMs, human liver microsomes; NTCP, Na^+-taurocholate cotransporting polypeptide; OAT, organic anion transporters; OCT, organic cation transporters; OATP, organic anion transporter polypeptides; P-gp, p-glycoprotein; UGTs, UDP-glucuronyltransferases

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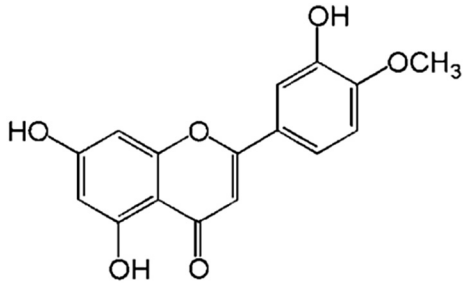

Fig. 1. Chemical structure of diosmetin.

et al., 2012).

A previous study showed that metabolic enzymes are the main factor inducing DDI. However, with an in-depth study of transporters, transporter-mediated DDI has gradually attracted the attention of researchers. Drug transporters include uptake and efflux transporters (Konig et al., 2013). Exogenous xenobiotics are transported into the liver due to uptake transporters, which affect the drug concentration in the organ and are thus associated with drug metabolism and excretion (Tsuji, 2006). Uptake transporters consist of the following four isoforms: organic anion transporters (OATs), organic cation transporters (OCTs), organic anion transporter polypeptides (OATPs) and Na^+-taurocholate cotransporting polypeptides (NTCPs). The FDA has provided recommendations to evaluate transporter-mediated drug interactions for new drug applications (Keogh, 2012). Therefore, it is important to explore whether the drug is an inhibitor or substrate of the transporter pathway.

Diosmetin (3′, 5, 7-trihydroxy-4′-methoxyflavone) (Fig. 1), a flavone found in plants belonging to the genus *Teucrium* (Lamiaceae) and in Portuguese olive leaves, is reported to exhibit a variety of pharmacological activities, such as anticancer, antimicrobial, antioxidative, estrogenic and anti-inflammatory effects (Kadifkova Panovska et al., 2005; Meirinhos et al., 2005). The flavone glycoside diosmin (3′,5,7-trihydroxy-4′-methoxy-methoxyflavone-7-ramnoglucoside) has clinically been used for treating venous tone and microcirculation and protecting capillaries. Diosmetin is the hydrolyzed and observed component of diosmin in the intestines after oral administration (Hnátek, 2015). Diosmetin is a potent inhibitor of CYP1A1 and CYP1B1, which can induce DDI, especially with concomitant administration of drugs metabolized by the same enzyme (Androutsopoulos et al., 2009). However, to the best of our knowledge, only a few studies have evaluated the effect of diosmetin on CYPs, and even fewer have evaluated its effects on UGTs or transporters. Due to the potential clinical application of diosmetin and adverse herb-drug interactions (HDIs) observed in many patients receiving conventional pharmacotherapy combined herbal medicines, this study investigated the possible effect of diosmetin on CYPs, UGTs and drug transporters to provide evidence for avoiding metabolic DDI (Levy et al., 2017).

2. Materials and methods

2.1. Chemicals and reagents

Diosmetin (purity > 98%, FY20331102) was obtained from Nantong Feiyu Biological Technology Co., Ltd. (Nantong, Jiangsu, China). Human liver microsomes (HLMs, Lot1210223) were purchased from XenoTech (Kansas, KS, USA). Dexamethasone, L-glutamine, Williams' medium E (WME), phenacetin, coumarin, dextromethorphan, amodiaquine, chlorzoxazone, testosterone, 7-hydroxycoumarin, paracetamol, hydroxyamfebutamone, 6β-hydroxytestosterone, 7-hydroxycoumarin-D-glucuronide, α-naphthoflavone, tranylcypromine, ticlopidine and quinidine were purchased from Sigma-Aldrich (St Louis, MO, USA). Amfebutamone, diclofenac and fluconazole were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). S-Mephenytoin, dextrorphan, *N*-deacetylation amodiaquine, 4-hydroxydiclofenac, 4-hydroxymephenytoin, 6-hydroxychlorzoxazone and N-3-benzylnirvanol were purchased from Toronto Research Chemicals (Toronto, Canada). Quercetin was purchased from Aladdin (Los Angeles, CA, USA). Ketoconazole was purchased from Civi Chem & Applications (Shanghai, China). Ham's F12 medium, penicillin, streptomycin, and insulin-transferrin-selenous solution (ITS-G) was purchased from Gibco (Carlsbad, CA, USA).

2.2. Animals

Male Sprague-Dawley rats (200 g to 300 g) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Animal care and treatment were performed strictly in accordance with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation with a 12/12 h light/dark cycle in colony room maintained at constant temperature (22 ± 1 °C) with air conditioning. All animals had free access to sterilized food and water. Experimental procedures were in compliance with the Guide for Care and Use of Laboratory Animals (NIH publication, revised 1996).

2.3. Primary human hepatocyte culture

Human liver tissue in this study was obtained from surgical resection of paracancerous tissue from hepatocellular carcinoma in patients who signed informed consent. The tissue acquisition protocol was in accordance with the requirements issued by the local ethical commission in China. Three batches of primary human hepatocytes (ZS006, ZS011 and FY016) were cultured. No.ZS006 hepatocytes was isolated from the liver of a Chinese woman aged 45. The donors of No.ZS011 and FY016 liver were Chinese men with 53 and 36 years old respectively.

Primary human hepatocytes were prepared with the classic two-step collagenase perfusion method (Godoy et al., 2013). In the first step, the liver pieces were perfused with EGTA-containing D-hank's solution to remove residual blood and warm up the tissue. The end of this perfusion step is indicated by a light-red color of the out-flowing perfusion solution. During step two of the perfusion process, cell–matrix contacts are destroyed by the digestion of ECM proteins with collagenase IV. Following the digestion procedure, the cannulae have to be pulled out quickly. BSA-containing stop solution is immediately poured over the tissue for enzyme inactivation to prevent the over-digestion of the liver tissue. The digested liver tissue is gently tweezed and cut into two halves with a scalpel without cutting through the capsule. Then tweeze the tissue gently and release the cells from the tissue into the surrounding stop solution. The cell suspension was collected with a serological pipette and passed through the prepared funnel with gauze into ice-cooled 50 mL tubes in order to eliminate tissue debris. The cells were centrifugated at 100 g at 4 °C for 5 min and resuspended in Williams' medium E (WME) and Ham's F-12 (1:1) medium supplemented with 5% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 6.25 μg/mL insulin, 6.25 μg/mL transferrin, 0.01 μM dexamethasone, and 2 mM L-glutamine for human hepatocyte culture. The cell number and viability was determined by the trypan blue exclusion assay. Hepatocyte viability should be higher than 80%. Cells were resuspended and seeded in collagen-coated 48-well plates at a concentration of 1.4×10^5 cells/well. After 4 h of incubation at 37 °C and 5% CO_2 in a humidified atmosphere, hepatocytes were overlaid with fresh medium containing matrigel (0.25 mg/mL).

2.4. Primary rat hepatocyte culture

Primary rat pepatocytes were prepared with the method of two-step collagenase perfusion *in situ* liver tissue (Godoy et al., 2013). In brief, rats were anesthetized with pentobarbital sodium (30 mg/kg) and abdominal cavity were opened by scalpel. During the two steps of