Assessment of Feng-Liao-Chang-Wei-Kang as a potential inducer of cytochrome P450 3A4 and pregnane X receptors

Yiqiang Xie[1], Yuhua Li[2], Mianqing Huang[3], Qing Wu[4], Qibing Liu[3], Junqing Zhang[3], Huiming Deng[3], Mi Liu[3] and Ling Huang[3]*

[1]The School of TCM of Hainan Medical University, Haikou, PR China
[2]Department of Pharmacy, the First Affiliated Hospital of Nanchang University, Nanchang, PR China
[3]School of Pharmaceutical Sciences of Hainan Medical University, Haikou, PR China
[4]Department of Pharmacy, the Affiliated Children's Hospital of Chongqing Medical University, Chongqing, PR China

Abstract: Feng-Liao-Chang-Wei-Kang (FLCWK), a traditional Chinese patent medicine, consists primarily of *Polygonum hydropiper and Daphniphyllum calycinum* roots. As a complex containing several kinds of flavonoids, FLCWK has the potential to impact the drug metabolism enzyme P450 3A4 (CYP3A4) and nuclear receptors. The purpose of this research was to probe the effects of FLCWK on CYP3A1, the homolog of CYP3A4 in rats, and to confirm whether FLCWK interferes with PXR and CAR-mediated transactivation of CYP3A4. The effects of FLCWK on *Cyp3a1* mRNA, catalytic activity levels, and protein expression in Sprague-Dawley (SD) rat liver tissues were examined using real-time PCR, western blotting, and high-performance liquid chromatography (HPLC) assays, respectively. The efficacy of PXR and CAR on CYP3A4 transcriptional activity were detected using luciferase reporter assays and further research of the impact of FLCWK on CYP3A4 gene expression mediated by the PXR pathway was examined by transient transfection of PXR siRNA. FLCWK significantly increased *Cyp3a1* mRNA, CYP3A1 activity, and protein expression levels in SD rats. FLCWK highly induced CYP3A4 luciferase activity mediated by PXR in PXR-CYP3A4 co-transfected cells. A siRNA-mediated drop-off in PXR expression greatly cut the effect of FLCWK on CYP3A4 mRNA expression in HepG2 cells. These findings show that FLCWK up-regulates CYP3A4 levels via the PXR pathway. This effect should be considered being applied in clinical use as FLCWK has the potential to interact with other drugs.

Keywords: Feng-Liao-Chang-Wei-Kang, Cytochrome P450 3A4, Cytochrome P450 3A1, Pregnane X receptor, herb-drug interaction.

INTRODUCTION

As a traditional Chinese patent medicine, Feng-Liao-Chang-Wei-Kang (FLCWK) is consisted of *Polygonum hydropiper and Daphniphyllum calycinum* roots (Zhang *et al*., 2011). Based on the clinical performance of FLCWK in the treatment of chronic superficial gastritis, ulcerative colitis, acute gastroenteritis, and mesenteric lymphadenitis (Mota *et al*., 2009; Tuñón *et al*., 2009), FLCWK has been designated as one of China's national protected traditional medicines. *Polygonum hydropiper* is known to contain sesquiterpenoids and flavonoids and has antioxidant, anti-inflammatory and antimicrobial properties (Tao *et al.,* 2016). *Daphniphyllum calycinum* has been shown to have potent antioxidant and anti-inflammatory properties as well and contains alkaloids and flavonoids (Gamez *et al.,* 1998).

According to our previous findings, flavonoids make up no less than 12% of FLCWK by weight. Flavonoids have demonstrated protective effects on the gastrointestinal tract through anti-inflammatory and antioxidant pathways (Chen *et al.,* 2016). Kaempferol and rutin are the major flavonoids present in FLCWK (Zhang *et al.,* 2011). Keampferol is known to alter MAPK/ERK signaling at several key locations as well as the PI3K/AKT pathway attenuating the risk of chronic conditions like inflammation and cancer (Chen & Chen 2013). Dietary rutin can cut the production of pro-inflammatory genes, for example, IL-6, interleukin-1β (IL-1β), and inducible nitric oxide synthase (iNOS) (Hosseinzadeh & Nassiri 2014).

Herb-drug interactions occur when compounds induce or inhibit drug-metabolizing enzymes, which can lead to adverse effects and compromise the efficacy of the drugs (Dai *et al.,* 2001). Cytochrome P450 3A4 (CYP3A4) is the most plentiful and significant isoform of the cytochrome P450 metabolizing enzymes in human intestine and liver. CYP3A4 is involved in 60% of drug metabolism and often plays a key role in drug interactions (Martínez *et al.,* 2007). As a family of ligand-activated transcription factors, nuclear receptors (NRs) have been identified as mediators of the drug-induced expression of CYP3A4. In these receptors, the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) are the primary mediators of CYP3A4 activity (Cheng *et al.,* 2011; Wonqanan *et al.,* 2014) as both play a part in

**Corresponding author:* e-mail: puer6@163.com

CYP3A4 transcriptional regulation following activation by xenobiotics (Waxman 1999).

Our previous study demonstrated the basic pharmacokinetic characteristics of FLCWK (Zhang *et al.,* 2011). However, the herb-drug interaction profile of FLCWK has yet to be studied. Yu *et al* have shown that the activities of CYP3A4 and NRs are affected by various flavonoid compounds including kaempferol and rutin (Yu *et al.,* 2011; Lehmann *et al.,* 1998), suggesting that FLCWK, as a complicated containing several kinds of flavonoids, has the potential to affect CYP3A4 and NRs and influence drug metabolism. Here, we evaluate the efficacy of FLCWK on CYP3A4, CYP3A1 (the homolog of CYP3A4 in rats), and the underlying mechanism of FLCWK's influence on PXR/CAR- mediated transactivation of CYP3A4.

MATERIALS AND METHODS

Cells and chemicals

The human liver cancer cell line HepG2 (The Chinese Academy of Sciences, Shanghai) was cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS), penicillin (100U/mL), and streptomycin (0.1 mg/mL) at 37°C with 5% CO_2. FLCWK was purchased from Guangdong Guoyitang Pharmaceutical Co. Ltd. (Guangzhou, China). Rifampicin (RIF), Dexamethasone （DXM), 6-(4-chloro phenyl) imidazo [2,1-b] [1,3] thiazole-5-carbaldehyde O-(3,4- dichloro benzyl) oxime (CITCO), midazolam (MDZ), and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, USA). The hPXR expression vector, hCAR2 expression plasmid, and pGL3-CYP3A4-XREM reporter plasmid were generously provided by Doctor Min Huang (Sun Yat-sen University, China) (Ling *et al.,* 2014). The dual-luciferase reporter assay system and the pRL Renilla Luciferase control reporter vectors were bought from Promega (Madison, USA). Anti-CYP3A1 polyclonal antibody and *β*-actin antibody were bought from Cell Signaling Technology (Danvers, USA). Anti-rabbit IG-HRP antibody was bought from R&D Systems (Minneapolis, USA).

Animals and treatmnets

Female Sprague-Dawley (SD) rats weighing 220±5g were bought from the Medical Experimental Animal Center of Hunan Province and stored at 22-24°C with a light/dark cycle of 12/12 h and relative humidity of 55%-60%. The rats can eat standard rodent chow and drink water freely at the Experimental Animal Center of Hainan Medical University. All animal experiments were performed according to the Regulations of the Experimental Animal Administration released by the Ministry of Science and Technology of the People's Republic of China. The design of the animal experiments was confirmed by the Experimental Animal Ethics Review Committee of Hainan Medical University.

FLCWK tablets were shattered and dissolved in PBS in at concentrations of 25, 50, and 100mg/mL. Rats were randomly separated into five groups and every group contained six rats. The first group was treated with DXM (10 mg/kg) as a positive control, the second group was treated with low-dose (0.25g/kg) FLCWK, the third was treated with medium-dose (0.5g/kg) FLCWK, the fourth with high-dose (1g/kg) FLCWK, and the fifth with a saline control. Each rat was orally given a 1 mL/100 g solution for ten consecutive days. After the final dose, rats were orally administered midazolam (MDZ) (15 mg/kg) and then blood samples (approximately 0.2mL each) were collected from the right jugular vein in preheparinized tubes pre-treatment and at 5, 10, 15, 30 and 45 min and at 1, 1.5, 2, 3, 4, 5 and 6 h post-treatment. Blood was then centrifuged immediately to separate 100μL of plasma. Rats were then sacrificed and liver tissues were harvested and separated. Plasma and liver tissue samples were kept at -80 °C until analysis.

Real-time PCR analysis

For each liver sample, total RNA was extracted by TRIzol reagent from 30mg liver tissue according to the manufacturer's guides and cDNA was synthesized for further analysis by a Primescript^{TM} RT Kit (Takara, Kyoto, Japan). The 20μL reaction mixture consisted of 10μL of SYBR Primix Ex Taq II (Takara, Kyoto, Japan), 1μL of the cDNA template, 8μL of RNase-free H_2O, and 0.5μL of each primer: CYP3A1 5'-TTCACCGTGATCC ACAGCA-3' and 3'-TGCTGCCCTTGTTCTCCTT-5'; CYP3A4 5'-TCAGCCTGGTGCTCCTCTATCTA T-3' and 3'-AAGCCCTTATGGTAGGACAAAATATTT-5'; PXR 5'- ATGGCAGTGTC TG GAACTAC-3' and 5'-CAGTTGACACAGCT CGAAAG-3'. The amplification conditions were as follows: a denaturation step was performed at 94°C for 60 s, followed by 45 cycles at 94 °C for 30 s, 58°C for 30s and 72°C for 30 s, then 95°C for 10 s, 65°C for 45s and 40°C for 60 s. Relative expression levels were figured by Data Assist Software version 3.0 (Applied Biosystems/Life Technologies) and the $2^{-\Delta\Delta ct}$ ways. Each treatment was represented by three replicates.

Western blotting analysis

Total protein was extracted from rat liver tissue samples after grinding with liquid nitrogen using 1% RIPA lysis buffer. The protein concentration was confirmed using a BCA protein assay kit. 40μg supernatant protein samples were separated by 8% SDS-PAGE and semi-dry blotted onto PVDF (polyvinylidene fluoride) membranes (Millipore, Bedford, MA, USA). After nonspecific membranes were blocked with 5% nonfat milk, the blots were incubated with a CYP3A1 and β-actin antibody overnight at 4°C and with anti-rabbit Ig-HRP antibody for

1h at room temperature. Protein levels were detected using the Supersignal® West Pico Chemiluminescent Substrate Kit (ThermoFisher Scientific; Waltham, MA, USA). Each treatment was represented by three replicates.

Analysis of CYP3A1 activity using HPLC for MDZ

A Waters Alliance 2695 HPLC apparatus was purchased from Waters Science and Technology Co. Ltd, TD. The chromatographic column was Diamonsil™ C_{18} (200 mm × 4.6 mm, 5 m), the mobile phase consisted of acetonitrile and 0.01 mol/ L potassium dihydrogen phosphate buffer solution (pH 5.0) (58:42). The flow rate was 1.0 mL/min and the detected wavelength was 220 nm.

Diphenytriazol-methanol solution was added into clean centrifuge tubes as an internal standard. After volatilizing the methanol to dryness, 0.1mL of plasma sample was added to the tubes. Next, 1mL of ethyl acetate extraction was added and the tubes were vortexed for three minutes followed by centrifugation at 13,000r/min for 5 min. Then 0.9 mL of the upper organic solvent was obtained for vacuum drying, the residue was dissolved with 50μL of the mobile phase, and 20μL of sample was evaluated at a wavelength of 220 nm.

Luciferase assay

HepG2 cells were seeded at 70%-80% confluence into 96-well plates at a density of 0.2×10^5 cells/well for 24h. Transfection mixtures were created with 50ng of hPXR or 50 ng of pcD-hCAR2 expression vector, 100 ng of CYP3A4-XREM luciferase reporter, and 15ng of pRL-TK Renilla luciferase reporter. After the transfection mixtures were incubated with Lipofectamine 2000 (Invitrogen, Carlsbad, USA) for 6 h, HepG2 cells were exposed to 10μM RIF or CITCO, which are known hPXR and hCAR activators, or FLCWK solution at 10, 20, and 40 mg/mL for 24h. Cells were then lysed, and Firefly and Renilla luciferase activity was measured using the dual-luciferase reporter assay system according to the manufacturer's instructions with a TD 20/20n single-tube luminometer (Promega, Madison, WI USA). The transfection efficiency was showed as the fold induction of Firefly to Renilla luciferase activities relative to the empty or vehicle control.

Transient transfection

In the RNAi experiment, HepG2 cells were plated in 6-well plates at a density of 5×10^6 and PXR siRNA or NC siRNA was transfected with siRNA transfection reagent (Roche, Branford, CT USA) according to the manufacturer's instructions. Primers for hPXR were as follows: 5′ - GCA CCT GCT GCT AGG GAA TA-3′ and 5′ - CTC CAT TGC CCC TCC TAA GT-3′. The target sequences of siPXR were as follows: 5′-GAUGGACGCUCAGAUGAAATT-3′ and 3′-UUUCAU CUGAGCGUCCAUCTT -5'. Cells were further incubated with 10 μM RIF or 10, 20, or 40mg/mL FLCWK for 72 h. After incubating, cells were harvested and total RNA was extracted and investigated by qPCR, (Ling 2013). Each treatment was represented by three replicates.

STATISTICAL ANALYSIS

Significant differences were confirmed using the unpaired Student's t-test or one-way analysis of variance (one-way ANOVA) followed by Dunnett's t-test with the software SPSS 13.0. $P<0.05$ was considered to indicate a statistically important difference.

RESULTS

FLCWK increases CYP3A1 expression in rats

As illustrated by qPCR (fig. 1), *Cyp3a1* mRNA expression level increased by 23.1-fold compared to the control group after DXM treatment for 10 days. FLCWK administration at dosages of 0.25, 0.5, and 1 g/mg increased *Cyp3a1* mRNA expression in rat livers by 3.93, 5.94, and 8.57-fold ($P<0.05$), respectively, showing that FLCWK had a vital effect on *Cyp3a1* mRNA expression in a dose-dependent manner.

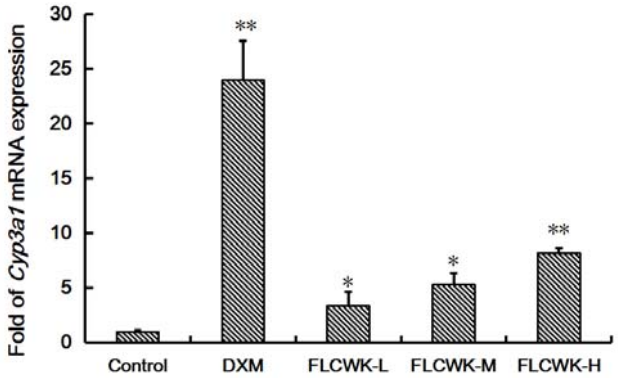

Fig. 1: Effects of FLCWK on CYP3A1 mRNA expression in rat liver. SD rats were administered saline (control group), DXM (10 mg/kg), 0.25g/kg (FLCWK-L), 0.5g/kg (FLCWK-M) or 1g/kg (FLCWK-H) FLCWK for ten consecutive days before liver tissues were removed for real-time PCR studies. Values are expressed as the *means* ± *SEM*, $n = 6$, *$P<0.05$, **$P<0.01$.

To investigate the effect of FLCWK on CYP3A1 protein expression, western blotting was performed on rat liver tissues. As is shown in fig. 2, CYP3A1 protein expression was significantly increased 3.38-fold after DXM administration and FLCWK at 0.5g/kg and 1 g/kg increased CYP3A1 protein expression by 1.89 and 2.44-fold ($P<0.05$), respectively.

FLCWK increases the catalytic activity of the CYP3A1 enzyme in SD rats

The concentration of MDZ, a probe used to determine CYP3A activity in vivo, was measured using HPLC to observe the inductive capacity of FLCWK on CYP3A1.

The average plasma concentration-time profile of MDZ is expressed in fig. 3 and table 1. Compared to control group, the $AUC_{0\text{-}360min}$ and the $AUC_{0\rightarrow\infty}$ of DXM group were significantly decreased (P<0.01), the AUC $_{0\rightarrow\infty}$ of MDZ of the rats administrated FLCWK for 10 days at dosages of 0.5g/kg and 1g/kg were decreased to 105.54±9.35 (P<0.05) and 92.00±7.68 (P<0.05), respectively. It suggests that FLCWK can significantly up-regulate the catalytic activity of CYP3A1.

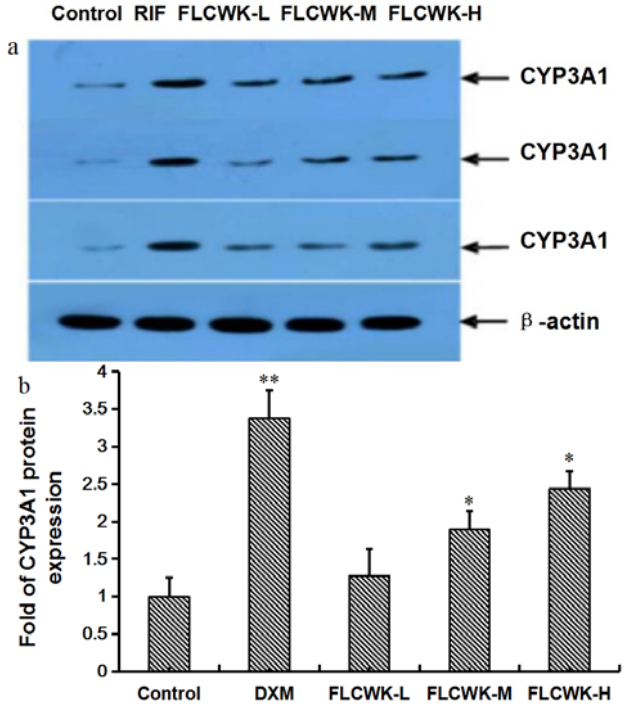

Fig. 2: Effects of FLCWK on CYP3A1 protein expression in SD rat liver. SD rats were administered saline (control group), DXM (10mg/kg), 0.25g/kg (FLCWK-L), 0.5g/kg (FLCWK-M) or 1g/kg (FLCWK-H) FLCWK for ten consecutive days before liver tissue was removed for western blotting. Values are expressed as the *means* ± *SEM*, n = 3, **P*<0.05, ***P*<0.01.

Effect of FLWCK on PXR/CAR-CYP3A4 luciferase reporter assay

To further understand the mechanism underlying the inductive effect of FLCWK on CYP3A4, a dual-luciferase reporter assay was utilized. The CYP3A4-XREM luciferase reporter plasmid and pRL-TK Renilla luciferase reporter were co-transfected together with hPXR or hCAR over-expression plasmids into HepG2 cells. FLCWK, at concentrations of 10, 20 and 40g/L, had negligible cytotoxicity towards native and transiently transfected HepG2 cells. Compared to the control group, CYP3A4 luciferase activity was significantly induced 6.67 or 5.69-fold by 10μM RIF or 10μM CITCO (fig. 4), indicating that the dual-luciferase reporter assay was successfully established.

When the CYP3A4-XREM reporter plasmid was co-transfected with the hPXR expression plasmid, FLCWK at concentrations of 20 and 40 g/L significantly increased CYP3A4 luciferase activity by 4.72 and 5.37-fold, respectively (P<0.01). However, FLCWK did not induce CYP3A4 luciferase activity when the CYP3A4-XREM reporter plasmid was co-transfected with the CAR expression plasmid, indicating that FLCWK upregulation of CYP3A4 is mediated by the PXR pathway rather than by the CAR pathway.

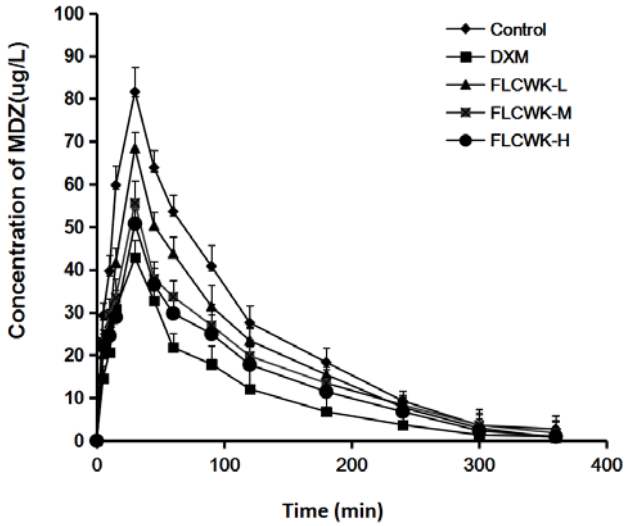

Fig. 3: Effects of FLCWK on CYP3A1 enzyme activity in SD rats. Plasma concentration-time curves of MDZ after saline, DXM (10mg/kg), 0.25g/kg (FLCWK-L), 0.5g/kg (FLCWK-M) or 1g/kg (FLCWK-H) treatment. Values are expressed as the *means* ± *SEM*, n = 6, **P*<0.05, ***P*< 0.01.

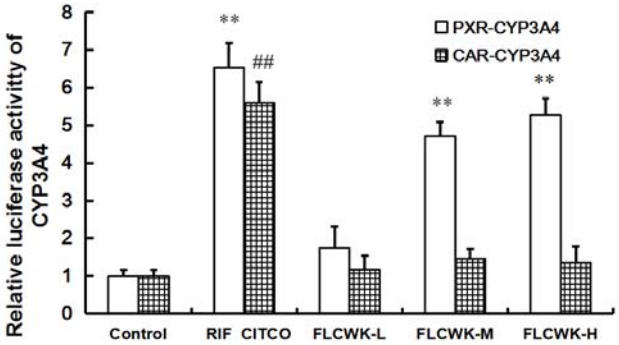

Fig. 4: The effect of FLCWK on CYP3A4 reporter gene construct transactivation in transiently transfected HepG2 cells. Values are expressed as the means of the fold increases in activity compared to the control vehicle-treated cells from more than three independent experiments. Values are expressed as the *means* ± *SEM*, n = 6, $^{\#\#}P$<0.01, $^{**}P$<0.01.

PXR is required for FLCWK regulation of CYP3A4 expression

To further investigate the up-regulation of CYP3A4 by

Table 1: The AUC of MDZ in SD rats.

Parameter	Control	DXM	FLCWK-L	FLCWK-M	FLCWK-H
$AUC_{0\text{-}360min}$ (mg/L·h)	148.14±10.21	65.29±5.16**	115.91±11.16	100.44±9.28*	88.71±7.55*
$AUC_{0\rightarrow\infty}$ (mg/L·h)	153.29±12.83	68.61±6.23**	118.52±15.27	105.54±9.35*	92.00±7.68*

SD rats were administered with saline, 10mg/kg DXM, 0.25g/kg (FLCWK-L), 0.5g/kg (FLCWK-M) or 1g/kg (FLCWK-H) FLCWK. Values are expressed as the means ± SEM, n = 6, *$P < 0.05$, **$P < 0.01$.

FLCWK through the PXR pathway, the effect of FLCWK on CYP3A4 mRNA levels in HepG2 cells with PXR knockdown was measured. fig. 5(a) shows that PXR was down-regulated by PXR siRNA. As is shown in fig. 5(b), after HepG2 cells were transfected with NC siRNA and treated with FLCWK at 20mg/mL, 40mg/mL for 72 h, *Cyp3a4* mRNA was increased 3.76 fold and 3.51-fold, respectively (P<0.05). However, the induction of CYP3A4 mRNA by FLCWK (40mg/mL) in PXR-silenced cells was significantly suppressed to 35.7% and 57.2% compared to the unsilenced PXR group (P<0.05), suggesting that FLWCK up-regulates CYP3A4 expression via PXR.

DISCUSSION

CYP3A4 is a major player in drug metabolism that may be influenced by a series of compounds. Changes in CYP3A4 activity can result in altered systemic drug concentrations and even toxicity (Yang *et al.,* 2012; Brantley *et al.,* 2013), so it is important to clarify how traditional Chinese medicines affect drug metabolism to improve the safety and efficacy of these medications (Hsin *et al.,* 2013; Fasinu *et al.,* 2012). In recent years, the effects of Chinese patent medicines on drug-metabolizing enzymes have become an increasingly important area of interest.

FLCWK is a popular traditional Chinese patent medicine with proven therapeutic effects on gastrointestinal diseases such as chronic superficial gastritis and ulcerative colitis. Several studies have shown that flavonoid compounds can affect the activity of CYP3A4 via the NR pathway (Cheng *et al.,* 2011). Kaempferol and rutin are the main effective components of FLCWK; Kaempferol has been reported to inhibit CYP3A expression in rats and further cell-based gene assays have revealed that kaempferol can activate the expression of PXR and CAR (Chang 2009; Yao *et al.,* 2010). Rutin can reduce the bioavailability of immunosuppressants by activating CYP3A4 and P-glycoprotein (Martínez *et al.,* 2007). Therefore, it is assumed that FLCWK can affect the expression of CYP3A4 and NRs and may therefore interfere with drug metabolism.

Rat liver tissue is an ideal model for observing the effects of FLCWK on *Cyp3a1* mRNA and protein expression due to its higher endogenous expression of many CYP enzymes (Li *et al.,* 2012; Choi *et al.,* 2010). In the present study, we demonstrated that *Cyp3a1* mRNA (the rat homolog for human *Cyp3A4*) and protein expression levels were significantly increased in rat liver tissues after FLCWK treatment. This suggests that the compounds have the potential to affect CYP3A1 activity during clinical application.

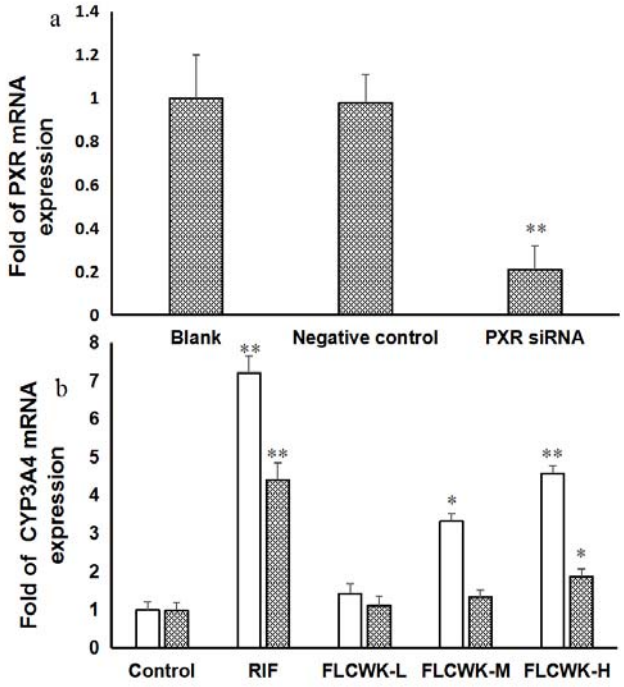

Fig. 5: Effects of FLCWK on CYP3A4 mRNA expression in in HepG2 cells. (a) HepG2 cells were transfected with negative control siRNA or PXR siRNAs. PXR mRNA levels were analyzed by real-time PCR. **P<0.01. (b) HepG2 cells were transfected with negative control or PXR siRNA and were treated with 10μM RIF or FLCWK for 72 h. The CYP3A4 mRNA expression was analyzed using real-time quantitative PCR. *P<0.05, **P<0.01 compared to control in NC siRNA transfected groups. Values are expressed as *means* ± *SEM*, n = 6.

To further confirm the inductive effect of FLCWK on CYP3A1, an HPLC assay was used to detect rat CYP3A1 catalysis activity. In an *in vivo* study, we found that CYP3A1 activity was increased after FLCWK treatment for ten consecutive days. The decreased AUC between 0 and 6 h after MDZ treatment observed in FLCWK-treated rats also indicated that FLCWK enhanced CYP3A1 activity. The DXM-treated rat model has been widely used to model the induction of CYP3A1 activity. In this

model, the plasma concentration of MDZ is dependent upon CYP3A1 activity. The observed effects of FLCWK on the AUC of MDZ are consistent with our results obtained from real-time PCR and Western blotting analyses, suggesting that FLCWK can significantly up-regulate the metabolic activity of CYP3A1.

PXR and CAR are the most important NRs regulating CYP3A4 transcription activity. A key event after ligand binding is the alteration of nuclear receptor conformation and the recruitment of regulator proteins that alter chromatin accessibility of target genes which form heterodimers to control gene transcription and subsequently trigger biological effects (Bogacz *et al.,* 2014; Watanabe *et al.,* 2013). We speculated that an alteration in CYP3A4 by FLCWK might be mediated by PXR and CAR and developed a dual-luciferase reporter assay to investigate the effects of FLCWK induction on CYP3A4 mRNA and protein levels and the enzymatic activities induced by the PXR or CAR signaling pathways. These results revealed that FLCWK could significantly transactivate CYP3A4 luciferase activity via the PXR pathway. However, FLCWK could not induce CYP3A4 luciferase activity when the CYP3A4-XREM reporter plasmid was co-transfected with the CAR expression plasmid, indicating that the PXR pathway rather than the CAR pathway is causally involved as a contributing mediator for the increased expression and catalytic activity induced by FLCWK.

To confirm the role of PXR in mediating FLCWK induction of CYP3A4 gene expression, we observed the effect of FLCWK on CYP3A4 when PXR was silenced. In the current research, we demonstrated that PXR knockdown with siRNA decreased the inductive effect of FLCWK on CYP3A4 expression. Further, FLCWK treatment increased CYP3A4 significantly in normal HepG2 cells but not in the PXR knockdown HepG2 cells. Therefore, PXR is essential for the FLCWK-mediated up-regulation of CYP3A4 expression in human hepatocytes.

In the current research, our purpose is to evaluate the effects of FLCWK on CYP3A4 and the underlying mechanism of action, which could provide proof for rational use of FLWCK. Due to the species differences between humans and rats, further studies focused on human hepatocytes and liver micro somes are required. In addition, more herb-drug interaction experiments should be done to provide more support for FLCWK rational use in the clinic.

CONCLUSION

In summary, we determined that FLCWK significantly increased CYP3A4 expression and activity via the PXR pathway. This effect may increase the probability of herb-drug interactions when FLCWK is either sequentially administered or combined with other drugs. These findings should be considered due to the specific herb-drug interactions that may occur during FLCWK administration.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (Grant No. 81760674).

REFERENCES

Bogacz A, Mrozikiewicz PM, Karasiewicz M, Bartkowiakwieczorek J, Majchrzycki M, Mikolajczak PL, Ozarowski M and Grzeskowiak E (2014). The Influence of Standardized Valeriana officinalis Extract on the CYP3A1 Gene Expression by Nuclear Receptors in *In Vivo* Model. *Biomed Res Int.,* **2014**(ID 819093): 16.

Brantley SJ, Graf TN, Oberlies NH and Paine MF (2013) A systematic approach to evaluate herb-drug interaction mechanisms: investigation of milk thistle extracts and eight isolated constituents as CYP3A inhibitors. *Drug Metab Dispos.,* **41**(9): 1662.

Waxman DJ (2013). P450 gene induction by structurally diverse xenochemicals: Central role of nuclear receptors CAR, PXR, and PPAR. *Arch. Biochem. Biophys.,* **369**: 11-23.

Chang TK (2009). Activation of Pregnane X Receptor (PXR) and Constitutive Androstane Receptor (CAR) by Herbal Medicines. *AAPS J.,* **11**(3): 590-601.

Chen AY and Chen YC (2013). A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. *Food Chem.,* **138**(4): 2099-2107.

Chen H, Pu J, Liu D, Yu W, Shao Y, Yang G, Xiang Z and He N (2016). Anti-Inflammatory and Antinociceptive Properties of Flavonoids from the Fruits of Black Mulberry (Morus nigra L.). *Plos One.,* **11**(4): e0153080.

Cheng J, Ma X and Gonzalez FJ (2011). Pregnane X receptor- and CYP3A4-humanized mouse models and their applications. *Br. J. Pharmacol.,* **163**(3): 461-468.

Choi YH, Lee U, Lee BK and Lee MG (2010). Pharmacokinetic interaction between itraconazole and metformin in rats: Competitive inhibition of metabolism of each drug by each other via hepatic and intestinal CYP3A1/2. *Br. J. Pharmacol.,* **161**(4): 815.

Dai D, Tang J, Rose R, Hodgson E, Bienstock RJ, Mohrenweiser HW and Goldstein JA (2001) Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. *J. Pharmacol. Exp. Ther.,* **299**(3): 825.

Fasinu PS, Bouic PJ and Rosenkranz B (2012). An overview of the evidence and mechanisms of herb-drug interactions. *Frontiers in Pharmacology,* **3**: 69.

Gamez EJC, Luyengi L, Sang KL, Zhu LF, Zhou BN,

Fong HHS, Pezzuto JM and Kinghorn AD (1998). Kinghorn AD. Antioxidant flavonoid glycosides from Daphniphyllum calycinum. *J. Nat. Prod.,* **61**(5): 706-708.

Hosseinzadeh H and Nassiriasl M (2014). Review of the protective effects of rutin on the metabolic function as an important dietary flavonoid. *J. Endocrinol. Invest,* **37**(9): 783.

Hsin-Hui Tsai H-WL, Ying-Hung Lu, Yi-Ling Chen and Gail B Mahady (2013). A Review of Potential Harmful Interactions between Anticoagulant/Antiplatelet Agents and Chinese Herbal Medicines. Plos One, **8**(5): e64255.

Huang L, Wu Q, Li YH, Wang YT and Bi HC (2013). PXR-Mediated Upregulation of CYP3A Expression by Herb Compound Praeruptorin C from Peucedanum praeruptorum Dunn. *Evid Based Complement Alternat Med.,* **2013**(6): 156574.

Ju A, Li YY, Qu Z and Li Q (2017). Impact of the Herbal Breviscapine on the Pharmacokinetics of Simvastatin in Rats: The Involvement of CYP3A4. PLoS One., **7**: e31312.

Keisuke W, Kaori S, Yuri T, Yasushi Y and Kouichi Y (2013). Dual roles of nuclear receptor liver X receptor α (LXRα) in the CYP3A4 expression in human hepatocytes as a positive and negative regulator. *Biochem Pharmacol.,* **86**(3): 428-436.

Martínez-Jiménez CP, Jover R, Donato MT, Castell JV and Gómez-Lechón MJ (2007). Transcriptional regulation and expression of CYP3A4 in hepatocytes. *Curr Drug Metab.,* **8**(2): 185.

Moore JT and Plc GS Lehmann JM *et al* (1998). The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.,* **102**: 1-8.

Mota KS, Dias GE, Pinto ME, Luizferreira A, Souzabrito AR, Hirumalima CA, Barbosafilho JM and Batista LM (2009). Flavonoids with gastroprotective activity. *Molecules.,* **14**(3): 979.

Tao J, Wei Y and Hu T (2016). Flavonoids of Polygonum hydropiper L. attenuates lipopolysaccharide-induced inflammatory injury via suppressing phosphorylation in MAPKs pathways. *BMC. Complement Altern. Med.,* **16**(1): 1-15.

Tunon M and Gonzalez-Gallego J (2009). Potential of Flavonoids as Anti-inflammatory Agents: Modulation of Pro-Inflammatory Gene Expression and Signal Transduction Pathways. *Curr Drug Metab.,* **10**(3): 256-271.

Udata C, Garzone PD, Gumbiner B, Joh T, Liang H, Liao KH, Williams JH and Meng X (2017). A Mechanism-Based Pharmacokinetic/Pharmacodynamic Model for Bococizumab, a Humanized Monoclonal Antibody Against Proprotein Convertase Subtilisin/Kexin Type 9, and Its Application in Early Clinical Development. *Acta Pharmacol Sin.,* **33**: 127-136.

Wonganan P, Jonssonschmunk K, Callahan SM, Choi JH and Croyle MA (2014). Evaluation of the HC-04 cell line as an in vitro model for mechanistic assessment of changes in hepatic cytochrome P450 3A during adenovirus infection. *Drug Metab Dispos.,* **42**(7): 1191.

Yao R, Yasuoka A, Kamei A, Kitagawa Y, Tateishi N, Tsuruoka N, Kiso Y, Sueyoshi T, Negishi M and Misaka T (2011). Dietary flavonoids activate the constitutive androstane receptor (CAR). *J. Agric. Food Chem.,* **58**(4): 2168.

Yu CP, Wu PP and Hou YC (2011). Quercetin and Rutin Reduced the Bioavailability of Cyclosporine from Neoral, an Immunosuppressant, through Activating P-Glycoprotein and CYP 3A4. *J. Agric. Food Chem.,* **59**(9): 4644-4648.

Zhang J, Liu X, Fu N, Liu M and Tan Y (2011). Systemic exposure of quercetin after administration of feng-liao-chang-wei-kang granules to rats. *J. Ethnopharmacol.,* **133**(2): 911.