Journal of Natural Medicines
https://doi.org/10.1007/s11418-018-1184-z

ORIGINAL PAPER

Effect of the active ingredient of *Kaempferia parviflora*, 5,7-dimethoxyflavone, on the pharmacokinetics of midazolam

Wataru Ochiai[1] · Hiroko Kobayashi[1] · Satoshi Kitaoka[1] · Mayumi Kashiwada[1] · Yuya Koyama[1] · Saho Nakaishi[1] · Tomomi Nagai[1] · Masaki Aburada[2] · Kiyoshi Sugiyama[3]

Received: 27 November 2017 / Accepted: 26 January 2018

Abstract
5,7-Dimethoxyflavone (5,7-DMF), one of the major components of *Kaempferia parviflora*, has anti-obesity, anti-inflammatory, and antineoplastic effects. On the other hand, in vitro studies have reported that it directly inhibits the drug metabolizing enzyme family cytochrome P450 (CYP) 3As. In this study, its safety was evaluated from a pharmacokinetic point of view, based on daily ingestion of 5,7-DMF. Midazolam, a substrate of CYP3As, was orally administered to mice treated with 5,7-DMF for 10 days, and its pharmacokinetic properties were investigated. In the group administered 5,7-DMF, the area under the curve (AUC) of midazolam increased by 130% and its biological half-life was extended by approximately 100 min compared to the control group. Compared to the control group, 5,7-DMF markedly decreased the expression of CYP3A11 and CYP3A25 in the liver. These results suggest that continued ingestion of 5,7-DMF decreases the expression of CYP3As in the liver, consequently increasing the blood concentrations of drugs metabolized by CYP3As.

Keywords *Kaempferia parviflora* · Cytochrome P450 · CYP3As · Midazolam · 5,7-Dimethoxyflavone · Pharmacokinetics

Introduction

The *Kaempferia parviflora* rhizome has been widely used as a traditional herbal medicine for over 1000 years in Thailand and Laos, where it is consumed as tea or liqueur. It is used for nutritional enhancement, recovery of physical strength, reduction of blood glucose levels, as well as for improving the function of the cardiovascular and digestive systems. In recent years, *K. parviflora* has been reported to possess many pharmacological properties, and has been used for various applications [1]. Its pharmacological actions include antineoplastic [2], cholinesterase inhibitory [3], antioxidant, anti-inflammatory [4], antispasmodic [5], nitrogen monoxide production inhibitory [6], anti-allergic [7], gastric ulcer improving [8], and vasodilatory [9] activities. It is also known for its ability to prevent various metabolic syndromes due to its anti-obesity activity [10–16] and improvement of muscle endurance [17] and cognitive dysfunction [18], and is used for the prevention of skin aging [19].

The *K. parviflora* extract has been found to contain 12 different kinds of flavonoids [4, 12, 15], of which 5,7-dimethoxyflavone (5,7-DMF) has been reported to possess anti-inflammatory, antineoplastic, and anti-obesity activities [4, 20, 21]. It has also been reported that upon oral ingestion of *K. parviflora*, 5,7-DMF is detected in the blood [4]. On the other hand, in vitro studies have reported the inhibitory effects of 5,7-DMF on the activity of cytochrome P450 (CYP) 3As, a drug metabolizing enzyme family [22, 23]. It is reported that drug metabolizing enzymes CYP3As are regulated by nuclear receptors, i.e., pregnane X receptor (PXR) and constitutive active/androstane receptor (CAR) [24, 25]. Both receptors were first characterized as xenosensing transcription factors regulating the induction of

Wataru Ochiai and Hiroko Kobayashi have contributed equally to this work.

✉ Wataru Ochiai
w-ochiai@hoshi.ac.jp

✉ Kiyoshi Sugiyama
sugiyama@hoshi.ac.jp

[1] Department of Clinical Pharmacokinetics, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

[2] Faculty of Pharmacy, Musashino University, 1-1-20 Shinmachi, Nishitokyo-shi, Tokyo 202-8585, Japan

[3] Department of Functional Molecule, Kinetics Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

Published online: 17 March 2018

xenobiotic metabolizing enzymes in response to exogenous stimuli.

In recent years, there has also been an increase in the consumption of *K. parviflora* as a health supplement in Japan. This report seeks to answer questions regarding the safety of *K. parviflora*. Therefore, to confirm its safety from a pharmacokinetic point of view, we investigated the influence of 5,7-DMF as a target drug on CYP3As in an in vivo experiment.

To examine the effect of 5,7-DMF on CYP3A, we first investigated whether the results of two studies performed under in vitro conditions could be replicated under in vivo conditions. The first involved investigating the effect of 10-day oral administration of 5,7-DMF on the metabolic activity of midazolam, a CYP3As substrate [26–28]. In the second, we investigated whether the expression of CYP3As was altered in the livers of mice administered 5,7-DMF for 10 days.

Materials and methods

Materials

5,7-DMF was purchased from Indofine Chemical Company, Inc. (Hillsborough, NJ, USA). 1′-Hydroxymidazolam-d_4 solution was purchased from Sigma-Aldrich (St. Louis, MO, USA). Midazolam was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Rabbit anti-rat CYP3A2 antibody was purchased from Nosan Corporation (Kanagawa, Japan). Anti-β actin antibody (ab8227) was purchased from Abcam (Tokyo, Japan). Anti-rabbit IgG, HRP-linked whole Ab donkey was purchased from GE Healthcare (Chalfont St. Giles, UK).

Animal handling

We purchased 6-week-old male ICR mice from Japan SLC, Inc. (Shizuoka, Japan). The mice were kept at room temperature (24 ± 1 °C) and 55 ± 5% humidity with 12 h of light (artificial illumination 08:00–20:00). Food and water were available ad libitum. Each animal was used only once. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, as adopted by the Committee on Animal Research at Hoshi University.

Preparation of solutions

The 5,7-DMF solution was prepared with reference to the method of Bei and An [29].

5,7-DMF was dissolved in dimethyl sulfoxide to prepare a stock solution of 20 mg/mL. Next, a 20-fold dilution of the stock solution (1.0 mg/mL) was prepared with a solvent containing 35% polyethylene glycol 400, 2% ethanol, and 63% water. A 50 mg/mL stock solution of midazolam was prepared with Tween as the solvent. Using physiological saline, a 50-fold dilution of this stock solution (1.0 mg/mL) was prepared as the midazolam solution.

Administration of 5,7-DMF solution and dissection

The 5,7-DMF solution was orally administered to mice once a day for 10 days. The midazolam solution was orally administered 10 min after the final administration of the 5,7-DMF solution. The dose of both solutions was 100 µL/10 g body weight of the mouse. The mice were chronologically laparotomized under isoflurane anesthesia following midazolam administration (5, 15, 30, 60, 120, 180, 240 and 300 min) and blood was collected from the middle cardiac vein. Phosphate-buffered saline (PBS) was perfused throughout the body, beginning in the heart to flush out the blood from the organs, and then the liver was excised. The excised liver was rapidly frozen under liquid nitrogen and stored at −80 °C.

Determination of the blood concentration of midazolam by liquid chromatography–mass spectrometry (LC–MS)

An equal volume of aqueous phosphoric acid solution was added to 100 µL of plasma separated from the blood and the mixture was vortexed for 1 min. Simultaneously, 100 ng of 1′-hyrdroxymidazolam-d_4 was spiked as an internal standard substance. The solution was centrifuged at 15,000×*g* RT for 5 min. The supernatant, in a volume of 190 µL, was added to the solid-phase extraction columns (Oasis® HLB PRiME 3cc, 60 mg; Waters, Milford, MA, USA). These columns were preconditioned with 1 mL of methanol and equilibrated with 500 µL of ultrapure water and 2% aqueous phosphoric acid. Washing was performed with 500 µL methanol: ultrapure water (5:95, v/v). Sample elution was carried out using 1 mL of an acetonitrile: methanol (90:10, v/v) preparation. The solvent was removed from the eluate by distillation under nitrogen perfusion at 50 °C. The eluate was redissolved in the mobile phase at the start of the gradient of 100 µL to prepare an LC–MS sample. A calibration curve was prepared by spiking a standard solution of midazolam to each sample of control and pretreating in the same manner. The quantitation range of the calibration curve was prepared in the range of maternal plasma 1.95–500 ng/mL.

LC–MS analysis was performed using a combination of an LC-20A high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) and LCMS-2010 (Shimadzu). The analytical column that was used was an XBridge C18 (Waters). The analysis was performed at a column temperature of 40 °C. Ultrapure water containing

0.1% formic acid was used as mobile phase A. Acetonitrile containing 0.1% formic acid was used as mobile phase B. The analysis was performed in binary gradient mode, as shown below [gradient condition (% of B); 0.00 min 10%, 10.00 min 70%, 12.00 min 70%, 12.01 min 10%, 16.00 min 10%]. The flow rate was constant at 0.2 mL/min.

For the interface, positive mode electrospray ionization was used. The nebulizer gas flow rate was set at 1.5 L/min. The CDL and heat block temperatures were 250 and 200 °C, respectively. The voltage of the detector was set at 1.5 V. Measurement was performed by the selected ion monitoring method based on an *m*/*z* value of 326 for midazolam, 346 for 1′-hyrdroxymidazolam-d_4 (internal standard).

The LCMSsolution Ver.3.40 (Shimadzu) was used for HPLC system control and MS chromatogram analysis.

Preparation of the CYP3As proteins

In total, 1.0×10^5 COS7 cells were dispensed into a 6-cm dish. After 24 h, a method similar to transfection was carried out to transfect each vector with 2 μg of the plasmid containing the CYP3As gene. Twenty-four hours later, the culture medium was suctioned and cells were washed with 1 mL of PBS (−) three times on ice. Three hundred microlitres of RIPA buffer (Tris–HCl, 25 mM; NP-40, 0.1%; NaCl, 150 mM; SDS, 0.1%; SDC, 0.1%; protease inhibitor cocktail, 1%) was added, and cells were resuspended in a 1.5-mL microtube. The microtube was then vortex-mixed and sonicated for 1 min each, centrifuged at 21,500×*g* for 10 min at 4 °C, and the supernatant was collected. This solution was used as the standard protein of each CYP3As species.

Preparation of microsomal fraction

One milliliter of dissecting buffer (sucrose, 300 mM; imidazole, 25 mM; EDTA, 1 mM; protease inhibitor cocktail, 1%) was added to the liver extracts and homogenized on ice. Homogenate (9000 g) was centrifuged for 20 min at 4 °C, and then 800 μL of the supernatant was collected, and centrifuged at 105,000×*g* for 1 h at 4 °C. After discarding the supernatant, an appropriate quantity (300–1000 μL) of RIPA buffer was added to the pellet, which was dispersed with a sonicator and used as the microsomal fraction sample.

Western blotting

An equivalent amount of sample buffer was added to the sample solution, mixed well, and boiled for 5 min at 95 °C. Furthermore, electrophoresis was performed on 10% polyacrylamide gel (5.0 g/lane, 110 min, 20 mA). The proteins were then electroblotted onto a polyvinylidene difluoride (PVDF) membrane and blocking (skim milk, 5%; 10 min) was performed. Thereafter, the PVDF membrane was placed in a primary antibody solution (anti-rat CYP3A2 Ab 1/10,000; rabbit anti-β-actin antibody 1/10,000) for 1 h at 25 °C. After washing well with TBS-Tween20, the membrane was placed in a second antibody solution (donkey anti-rabbit IgG-HRP Ab 1/10,000) for 1 h at 25 °C. After washing the PVDF membrane with TBS-Tween20, it was exposed to ECL prime Western blot detection reagents. An image of the PVDF membrane was captured with a cooled CCD camera.

Quantitative-PCR

RNA was extracted from the mouse liver using the TRI reagent. A high-capacity cDNA synthesis kit was used to synthesize cDNA from 1 μg of RNA. Target gene expression was analyzed with real-time PCR. The primers were used as Table 1. Real-time PCR was conducted at a denature-action temperature of 95 °C for 15 s, an annealing temperature of 56 °C for 30 s, and an elongation temperature of 72 °C for 30 s. The mRNA gene expression levels were normalized to β-actin gene expression levels.

Pharmacokinetics analysis

The analytical software Numeric Analysis Program for Pharmacokinetics (NAPP) was used to calculate various pharmacokinetic parameters [30].

Statistics

Experimental values are expressed as mean ± standard deviation. Outliers were calculated using the Smirnov–Grubbs outliers test. Significant differences were calculated using Student's *t* test.

Results

Effect of 5,7-DMF on body weight and liver weight

Either 5,7-DMF (10 mg/kg/day) or physiological saline was orally administered by feeding needle to mice once a day for 10 days. We also measured the weight of animals in both groups daily. The results indicated no change in body weight between the control group and the 5,7-DMF-administered group (Fig. 1a). Since the total metabolic activity of CYP3A is affected by the size of the liver, the ratio of liver size to body weight was also measured. These results indicated that the dose of 5,7-DMF used in this study did not induce liver injury in the mice (Fig. 1b).

Table 1 Primer sequences of mouse mRNA

Targets	Primer (5′–3′)	Product size (bp)	NCBI reference sequence
IL-1β			
Forward	TGG CCT TGG GCC TCA AAG	93	NM_008361
Reverse	GCT TGG GAT CCA CAC TCT C		
IL-6			
Forward	TAG TCC TIC CTA CCC CAA TTT CC	76	NM_031168
Reverse	TTG GTC CTT AGC CAC TCC TTC		
TNF-α			
Forward	AAG CCT GTA GCC CAC GTC GTA	122	NM_013693
Reverse	GGC ACC ACT AGT TGG TTG TCT TTG		
β-Actin			
Forward	GAG CGC AAG TAC TCT GTG TG	97	NM_007393
Reverse	CGG ACT CAT CGT ACT CCT G		

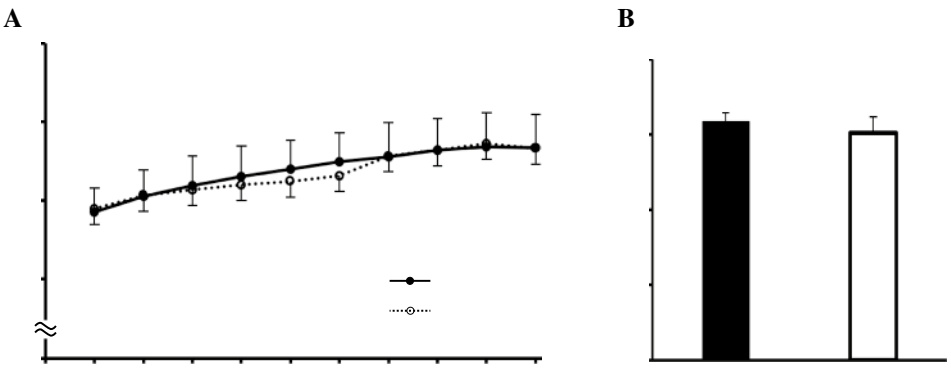

Fig. 1 Effect of 5,7-DMF on body weight and liver weight. Measurement of the value of the change in body weight divided by body weight (**a**) and change in liver weight divided by body weight (**b**) over a 10-day period of oral administration of 5,7-DMF or vehicle

Influence of 5,7-DMF on the pharmacokinetics of midazolam

Midazolam is representative of drugs that are metabolized by CYP3As. Therefore, midazolam (10 mg/kg/day) was administered by feeding needle to mice that had been orally administered 5,7-DMF for 10 days. The effect of 5,7-DMF administration on the metabolism of midazolam was then investigated. The results demonstrated a maximum blood concentration (C_{max}) of 18 ng/mL of midazolam in the control group, whereas in the group administered 5,7-DMF, a C_{max} of approximately 100 ng/mL was observed (Fig. 2a, b). In addition, a 1.3-fold increase in AUC was observed in the 5,7-DMF-administered group compared to the control group. Furthermore, the biological half-life ($t_{1/2}$) increased to 290 min in the 5,7-DMF-administered group compared to 187 min in the control group (Table 2).

Effect of 5,7-DMF on the liver protein expression levels of CYP3As

5,7-DMF administration resulted in an increase in the AUC and a prolongation of the $t_{1/2}$ of midazolam. We hypothesized that this was due to a change in the expression levels of CYP3A in the liver. Hence, using the Western blot method we analyzed the expression levels of CYP3A11 and CYP3A25 in the liver. The results demonstrated a significant reduction in liver protein expression levels of CYP3A11 and CYP3A25 in the 5,7-DMF-administered group compared to the control group (Fig. 3).

Effect of 5,7-DMF on inflammatory cytokines in the liver

It is well known that hypersecretion of inflammatory cytokines decreases the expression of CYP3A. Therefore,

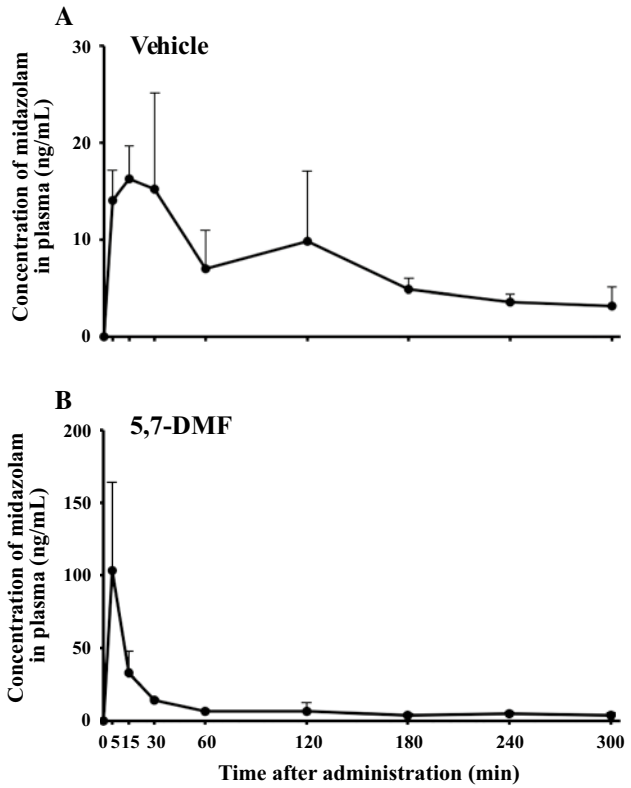

Fig. 2 Effect of 5,7-DMF on the pharmacokinetics of midazolam. Midazolam at 10 mg/kg body weight was orally administered to mice that had been orally administered 5,7-DMF (10 mg/kg/day) (**b**) or vehicle (**a**) for 10 days. The midazolam concentration in the plasma 300 min immediately after administration was determined by LC–MS. The results are reported as median ± standard deviation ($n = 5$). Significant differences were calculated using Student's *t*-test

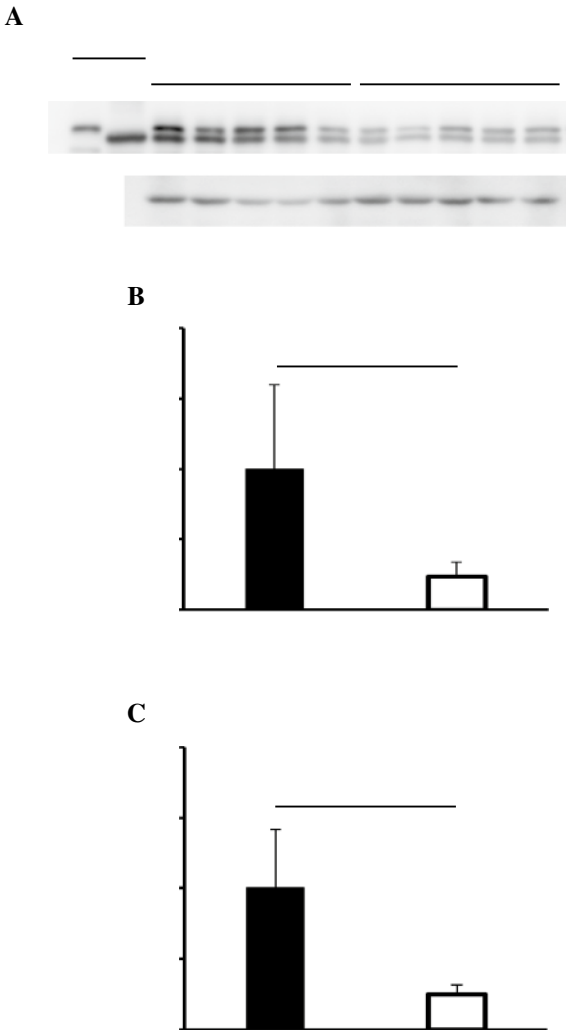

Fig. 3 Effect of 5,7-DMF on the expression level of CYP3A in the liver. After oral administration of 5,7-DMF ($n = 5$) or vehicle ($n = 5$) for 10 days, a liver microsome fraction was prepared. For the expression analysis of CYP3As, 1 ng of the microsome fraction was separated by SDS-PAGE followed by the Western blot method using anti-CYP3A2 antibodies. The band densities of CYP3A11 and CYP3A25 in the Western blot were then individually quantified by NIH Image. The results are reported as median ± standard deviation ($n = 5$). Standard proteins of CYP3A11 and CYP3A25 were applied as markers to lanes 1 and 2

Table 2 Pharmacokinetic parameters

Samples	$AUC_{0–300}$ (ng min/mL)	$t_{1/2}$ (min)
Control	2124	187
5,7-DMF	2696	290

Changes in the pharmacokinetic parameters during administration of 5,7-DMF. The AUC and $t_{1/2}$ were calculated using NAPP based on the blood concentration of midazolam in Fig. 2

we examined the mRNA expression levels of inflammatory cytokines in the liver. There were no differences in the expression levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) between the control and 5,7-DMF-administered group (Fig. 4). These results confirm that 5,7-DMF ingestion does not induce inflammation of the liver.

Discussion

A study of the safety of 5,7-DMF, the main component responsible for the pharmacological activity of *K. parviflora*, was performed focusing on pharmacokinetics.

In this study, 5,7-DMF was orally administered for 10 days at a concentration which did not produce toxicity as evaluated by mouse body and liver weight analysis, and the effect on midazolam pharmacokinetics was analyzed. An increase in C_{max} and AUC and a marked increase in $t_{1/2}$ were

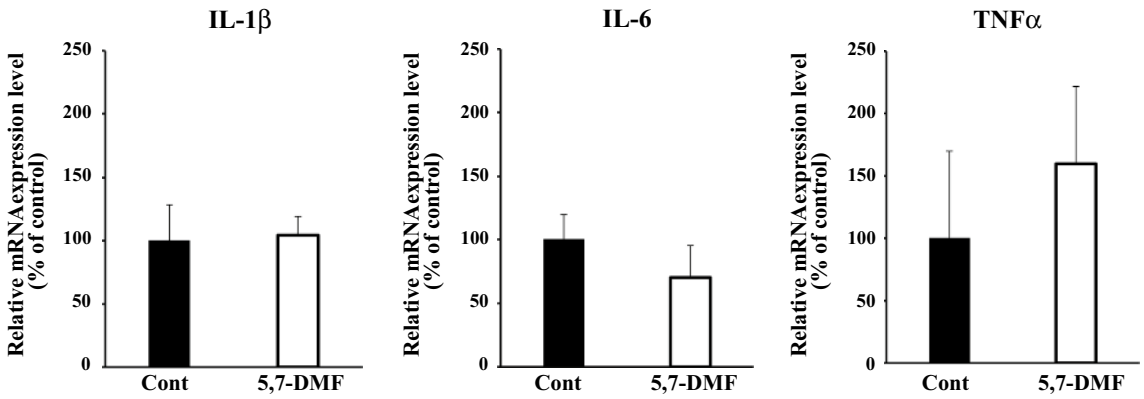

Fig. 4 Changes in the expression of liver inflammatory cytokines. Following oral administration of 5,7-DMF or vehicle for 10 days, the liver was excised, total RNA adjusted, and the expression level of inflammatory cytokine mRNA was measured by real-time PCR

observed in the group administered 5,7-DMF compared to the control group. Midazolam is a sedative-hypnotic drug that is metabolized by CYP3A. These results indicate that regular ingestion of 5,7-DMF will result in increased bioavailability of midazolam. Hence, careful attention will have to be paid to the subsequent appearance of adverse effects such as apnea and ventricular tachycardia.

There are various molecular species of mouse CYP3As, with various characteristics [24, 31]. In this study, we assessed CYP3A11 and CYP3A25. These molecules are the major molecules of mouse CYP3As. Mouse CYP3A11 is homologous to human CYP3A4 and is constitutively expressed in the liver of adult mice, where it plays a central role in drug metabolism [32, 33]. CYP3A25 is constitutively expressed in the liver of adult mice and is involved in metabolizing drugs and xenobiotic substances [34, 35]. We have shown that the expression levels of CYP3A11 and CYP3A25 are high in the adult mouse liver [36]. The main cause of the change in the pharmacokinetics of midazolam in the 5,7-DMF-administered group was a reduction in the expression levels of the drug-metabolizing liver enzymes. Therefore, this hypothesis was investigated. The results demonstrated a reduction in CYP3A11 and CYP3A25 expression levels in the 5,7-DMF-administered group compared to the control group (Fig. 4).

Next, we investigated the cause of the reduction in the expression level of CYP3As molecular species. Since an increase in inflammatory cytokines is known to decrease the expression of CYP3As, the expression levels of inflammatory cytokines at the time of administration of 5,7-DMF were assessed [25, 37]. No change in the expression levels of IL-1β, IL-6, and TNF-α was observed after 5,7-DMF administration. This suggests that the decrease in the expression of CYP3A11 and CYP3A25 by 5,7-DMF administration occurs via other mechanisms. These mechanisms may involve the direct or indirect inhibition of the expression of nuclear receptors, PXR or CAR, or nuclear translocation [25]. This issue remains unresolved and further analysis will be necessary in the future.

In this study, we investigated the effect of 5,7-DMF on the expression level of CYP3As in the liver with midazolam as the target drug, assuming that 5,7-DMF was ingested on a daily basis. The results demonstrated that long-term ingestion of 5,7-DMF reduces the expression of CYP3A. Currently, 40% of widely used medicines are substrates of CYP3A. Therefore, it is necessary to carefully consider the results of the current study keeping in mind cases of chronic consumption of *K. parviflora* as a health supplement. The effects of 5,7-DMF from *K. parviflora*, i.e., increase in the blood concentrations and biological half-lives may be observed with drugs that are substrates of CYP3As. Since CYP families other than CYP3As are also responsible for metabolism of many pharmaceuticals, it is necessary to investigate the influence of 5,7-DMF and other active constituents in health supplements, on the expression levels and inhibitory actions of these enzymes in vivo in the future.

Acknowledgements We thank Mr. Mashu Ariumi, Mr. Seiya Takano, Mr. Shohei Harada, Ms. Natsumi Ikegaya, Ms. Haruka Ishihara, Ms. Ayumi Usuda, Mr. Reo Kainuma, Ms. Hazuki Taguchi, Ms. Mitsuki Takeda and Ms. Yumi Miyahara (Department of Clinical Pharmacokinetics, Hoshi University) for their technical assistance. This work was supported by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities 2014–2018 (MEXT, Grant S1411019). We would like to thank Editage (www.editage.jp) for English language editing.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Saokaew S, Wilairat P, Raktanyakan P, Dilokthornsakul P, Dhippayom T, Kongkaew C, Sruamsiri R, Chuthaputti A, Chaiyakunapruk N (2016) Clinical effects of Krachaidum (*Kaempferia parviflora*): a systematic review. J Evid Based Complementary Altern Med. https://doi.org/10.1177/2156587216669628
2. Potikanond S, Sookkhee S, Na Takuathung M, Mungkornasawakul P, Wikan N, Smith DR, Nimlamool W (2017) *Kaempferia parviflora* extract exhibits anti-cancer activity against HeLa cervical cancer cells. Front Pharmacol 8:630
3. Sawasdee P, Sabphon C, Sitthiwongwanit D, Kokpol U (2009) Anticholinesterase activity of 7-methoxyflavones isolated from *Kaempferia parviflora*. Phytother Res 23:1792–1794
4. Kobayashi H, Suzuki R, Sato K, Ogami T, Tomozawa H, Tsubata M, Ichinose K, Aburada M, Ochiai W, Sugiyama K, Shimada T (2017) Effect of *Kaempferia parviflora* extract on knee osteoarthritis. J Nat Med. https://doi.org/10.1007/s11418-017-1121-6
5. Wattanapitayakul SK, Suwatronnakorn M, Chularojmontri L, Herunsalee A, Niumsakul S, Charuchongkolwongse S, Chansuvanich N (2007) *Kaempferia parviflora* ethanolic extract promoted nitric oxide production in human umbilical vein endothelial cells. J Ethnopharmacol 110:559–562
6. Wattanapitayakul SK, Chularojmontri L, Herunsalee A, Charuchongkolwongse S, Chansuvanich N (2008) Vasorelaxation and antispasmodic effects of *Kaempferia parviflora* ethanolic extract in isolated rat organ studies. Fitoterapia 79:214–216
7. Tewtrakul S, Subhadhirasakul S, Kummee S (2008) Anti-allergic activity of compounds from *Kaempferia parviflora*. J Ethnopharmacol 116:191–193
8. Rujjanawate C, Kanjanapothi D, Amornlerdpison D, Pojanagaroon S (2005) Anti-gastric ulcer effect of *Kaempferia parviflora*. J Ethnopharmacol 102:120–122
9. Mekjaruskul C, Sripanidkulchai B (2015) Pharmacokinetic interaction between *Kaempferia parviflora* extract and sildenafil in rats. J Nat Med 69:224–231
10. Akase T, Shimada T, Terabayashi S, Ikeya Y, Sanada H, Aburada M (2011) Antiobesity effects of *Kaempferia parviflora* in spontaneously obese type II diabetic mice. J Nat Med 65:73–80
11. Horikawa T, Shimada T, Okabe Y, Kinoshita K, Koyama K, Miyamoto K, Ichinose K, Takahashi K, Aburada M (2012) Polymethoxyflavonoids from *Kaempferia parviflora* induce adipogenesis on 3T3-L1 preadipocytes by regulating transcription factors at an early stage of differentiation. Biol Pharm Bull 35:686–692
12. Kobayashi H, Horiguchi-Babamoto E, Suzuki M, Makihara H, Tomozawa H, Tsubata M, Shimada T, Sugiyama K, Aburada M (2016) Effects of ethyl acetate extract of *Kaempferia parviflora* on brown adipose tissue. J Nat Med 70:54–61
13. Matsushita M, Yoneshiro T, Aita S, Kamiya T, Kusaba N, Yamaguchi K, Takagaki K, Kameya T, Sugie H, Saito M (2015) *Kaempferia parviflora* extract increases whole-body energy expenditure in humans: roles of brown adipose tissue. J Nutr Sci Vitaminol (Tokyo) 61:79–83
14. Okabe Y, Shimada T, Horikawa T, Kinoshita K, Koyama K, Ichinose K, Aburada M, Takahashi K (2014) Suppression of adipocyte hypertrophy by polymethoxyflavonoids isolated from *Kaempferia parviflora*. Phytomedicine 21:800–806
15. Shimada T, Horikawa T, Ikeya Y, Matsuo H, Kinoshita K, Taguchi T, Ichinose K, Takahashi K, Aburada M (2011) Preventive effect of *Kaempferia parviflora* ethyl acetate extract and its major components polymethoxyflavonoid on metabolic diseases. Fitoterapia 82:1272–1278
16. Yoshino S, Kim M, Awa R, Kuwahara H, Kano Y, Kawada T (2014) *Kaempferia parviflora* extract increases energy consumption through activation of BAT in mice. Food Sci Nutr 2:634–637
17. Toda K, Hitoe S, Takeda S, Shimoda H (2016) Black ginger extract increases physical fitness performance and muscular endurance by improving inflammation and energy metabolism. Heliyon 2:e00115
18. Welbat JU, Chaisawang P, Chaijaroonkhanarak W, Prachaney P, Pannangrong W, Sripanidkulchai B, Wigmore P (2016) *Kaempferia parviflora* extract ameliorates the cognitive impairments and the reduction in cell proliferation induced by valproic acid treatment in rats. Ann Anat 206:7–13
19. Park JE, Woo SW, Kim MB, Kim C, Hwang JK (2017) Standardized *Kaempferia parviflora* extract inhibits intrinsic aging process in human dermal fibroblasts and hairless mice by inhibiting cellular senescence and mitochondrial dysfunction. Evid Based Complementary Altern Med 2017:6861085
20. Li H, Zhang X, Wang W (2017) Anticancer activity of 5, 7-dimethoxyflavone against liver cancer cell line HEPG2 involves apoptosis, ROS generation and cell cycle arrest. Afr J Tradit Complement Altern Med 14:213–220
21. Song Y, Kim MB, Kim C, Kim J, Hwang JK (2016) 5,7-Dimethoxyflavone attenuates obesity by inhibiting adipogenesis in 3T3-L1 adipocytes and high-fat diet-induced obese C57BL/6J mice. J Med Food 19:1111–1119
22. Mekjaruskul C, Jay M, Sripanidkulchai B (2012) Modulatory effects of *Kaempferia parviflora* extract on mouse hepatic cytochrome P450 enzymes. J Ethnopharmacol 141:831–839
23. Walle UK, Walle T (2007) Bioavailable flavonoids: cytochrome P450-mediated metabolism of methoxyflavones. Drug Metab Dispos 35:1985–1989
24. Anakk S, Kalsotra A, Kikuta Y, Huang W, Zhang J, Staudinger JL, Moore DD, Strobel HW (2004) CAR/PXR provide directives for Cyp3a41 gene regulation differently from Cyp3a11. Pharmacogenomics J 4:91–101
25. Yang J, Hao C, Yang D, Shi D, Song X, Luan X, Hu G, Yan B (2010) Pregnane X receptor is required for interleukin-6-mediated down-regulation of cytochrome P450 3A4 in human hepatocytes. Toxicol Lett 197:219–226
26. Ghosal A, Satoh H, Thomas PE, Bush E, Moore D (1996) Inhibition and kinetics of cytochrome P4503A activity in microsomes from rat, human, and cdna-expressed human cytochrome P450. Drug Metab Dispos 24:940–947
27. Maenpaa J, Hall SD, Ring BJ, Strom SC, Wrighton SA (1998) Human cytochrome P450 3A (CYP3A) mediated midazolam metabolism: the effect of assay conditions and regioselective stimulation by alpha-naphthoflavone, terfenadine and testosterone. Pharmacogenetics 8:137–155
28. Reves JG, Fragen RJ, Vinik HR, Greenblatt DJ (1985) Midazolam: pharmacology and uses. Anesthesiology 62:310–324
29. Bei D, An G (2016) Pharmacokinetics and tissue distribution of 5,7-dimethoxyflavone in mice following single dose oral administration. J Pharm Biomed Anal 119:65–70
30. Hisaka A, Sugiyama Y (1998) Analysis of nonlinear and nonsteady state hepatic extraction with the dispersion model using the finite difference method. J Pharmacokinet Biopharm 26:495–519
31. Nelson DR, Zeldin DC, Hoffman SM, Maltais LJ, Wain HM, Nebert DW (2004) Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. Pharmacogenetics 14:1–18
32. Li Y, Cui Y, Hart SN, Klaassen CD, Zhong XB (2009) Dynamic patterns of histone methylation are associated with ontogenic expression of the Cyp3a genes during mouse liver maturation. Mol Pharmacol 75:1171–1179
33. Sakuma T, Takai M, Endo Y, Kuroiwa M, Ohara A, Jarukamjorn K, Honma R, Nemoto N (2000) A novel female-specific member of the CYP3A gene subfamily in the mouse liver. Arch Biochem Biophys 377:153–162

34. Dai D, Bai R, Hodgson E, Rose RL (2001) Cloning, sequencing, heterologous expression, and characterization of murine cytochrome P450 3a25*(Cyp3a25), a testosterone 6beta-hydroxylase. J Biochem Mol Toxicol 15:90–99
35. Peng L, Yoo B, Gunewardena SS, Lu H, Klaassen CD, Zhong XB (2012) RNA sequencing reveals dynamic changes of mRNA abundance of cytochromes P450 and their alternative transcripts during mouse liver development. Drug Metab Dispos 40:1198–1209
36. Kitaoka S, Hatogai J, Iimura R, Yamamoto Y, Oba K, Nakai M, Kusunoki Y, Ochiai W, Sugiyama K (2018) Relationship between low midazolam metabolism by cytochrome P450 3A in mice and the high incidence of birth defects. J Toxicol Sci 43:65–74
37. Zhao M, Xiaofei L, Gang C, Wei L, Jing X, Gang H, Ruini C, Rui N, Wei S, Jian Y, Bingfang Y (2012) DEC1 binding to the proximal promoter of CYP3A4 ascribes to the downregulation of CYP3A4 expression by IL-6 in primary human hepatocytes. Biochem Pharmacol 84:701–711