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Research Paper

A herbal-drug interaction study of keishi-bukuryo-gan, a traditional herbal preparation used for menopausal symptoms, in healthy female volunteers

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Keywords
cytochrome P450; drug-metabolizing enzyme; herbal-drug interaction; Kampo medicine; menopausal symptoms

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Abstract

Objectives Many patients use herbal medicines to relieve menopausal symptoms. Keishi-bukuryo-gan contains five herbal components, and has been used for treating hypermenorrhoea, dysmenorrhoea and menopausal symptoms in Asian countries. In this study, we investigated the potential herb–drug interactions of keishi-bukuryo-gan in healthy female subjects.

Methods Thirty-one healthy females (20–27 years) were studied to evaluate their baseline activity of cytochrome P450 (CYP) 1A2, CYP2D6, CYP3A, xanthine oxidase (XO) and *N*-acetyltransferase 2 (NAT2) based on the urinary metabolic indices of an 8-h urine sample collected after a 150-mg dose of caffeine and a 30-mg dose of dextromethorphan, and also the urinary excretion ratio of 6β-hydroxycortisol to cortisol. Thereafter, the subjects received 3.75 g of keishi-bukuryo-gan twice daily for seven days, and underwent the same tests on post-dose day 7.

Key findings The geometric mean phenotypic index for CYP1A2 significantly decreased by 16% on day 7 compared with the baseline ($P = 0.026$). Keishi-bukuryo-gan did not alter the indices for CYP2D6, CYP3A, XO and NAT2.

Conclusions Keishi-bukuryo-gan may inhibit the activity of CYP1A2, which is predominantly involved in oestrogen metabolism. However, TJ-25 is unlikely to participate in herb–drug interactions involving medications predominantly metabolized by CYP2D6, CYP3A, XO and NAT2.

Introduction

Many patients worldwide use herbal products, including herbal supplements and traditional Chinese medicine formulations, in combination with prescribed medications.[1–3] One important implication of the global increase in herbal product usage is that the probability of herb–drug interactions has increased.[1–4]

In Japan, Kampo medicine, which originated from Chinese herbal medicines, has become an attractive alternative source of medicinal treatment for chronic diseases, with the potential to treat patients holistically by supporting the patient's own healing power.[5] More than 100 Kampo medicines have been approved as prescription drugs by the Ministry of Health, Labour and Welfare of Japan for the treatment of a wide variety of conditions, but so far little information about the potentially dangerous herb–drug interactions has been reported.

Many female patients are now using herbal medicines to relieve menopausal symptoms, such as hot flashes and night sweating.[3,6] In Japan, keishi-bukuryo-gan (Chinese name: Gui-Zhi-Fu-Ling-Wan) is one of the most frequently used Kampo medicines and it is widely accepted in Japan as an effective alternative treatment for hypermenorrhoea, dysmenorrhoea and menopausal symptoms.[7,8] The pharmacologically active ingredients of keishi-bukuryo-gan are shown in Table 1. Both in-vitro and animal studies suggest that several components of keishi-bukuryo-gan can affect the

Table 1 The composition of keishi-bukuryo-gan and the reported effects of the components on cytochrome P450 (CYP) 1A2, 2D6, 3A, xanthine oxidase (XO) and *N*-acetyltransferase (NAT2) activity reported in the literature

Herbal component	Species	Composition (% w/w)	Main active ingredients	Effects of active ingredients on enzyme activity (species)
Cinnamon bark	*Cinnamomum cassia* Blume	20	*O*-methoxycinnamaldehyde Unknown ingredients Procyanidin B2, epicatechin	CYP1A2 ↓ (rat) [9] CYP3A ↓ (rat) [10] CYP3A ↓ (human) [11]
Peony root	*Paeonia lactiflora* Pallas	20	Paeoniflorin Pentagalloylglucose	XO ↓ (cow) [12]
Peach kernel	*Prunus persica* Batch	20	Amygdalin Prunasin Emulsin	
Poria Sclerotium	*Poria cocos* Wolf	20	Unknown ingredients Eburicoic acid Ergosterol Pachyman	CYP3A ↑ (human) [13]
Moutan bark	*Paeonia suffruticosa* Andrews	20	Pentagalloylglucose Paeonol Paeonoside, Paeonolide, Apiopaeonoside Paeoniflorin, Oxypaeoniflorin	XO ↓ (cow) [12] NAT ↑ (human) [14]

activity of drug-metabolizing enzymes, such as cytochrome P450 (CYP) 1A2 and CYP3A (Table 1). However, there are no available data about the interactions between keishi-bukuryo-gan and other drugs.

As a safe and non-invasive test, urinary caffeine metabolic ratios have been widely used for assessing CYP1A2, xanthine oxidase (XO) and *N*-acetyltransferase 2 (NAT2) activity in herb–drug interaction screening surveys.[15–17] Dextromethorphan is metabolized in humans to dextrorphan by CYP2D6 through *O*-demethylation.[18] The urinary molar ratio of dextromethorphan to dextrorphan (i.e. dextromethorphan/dextrorphan), after a single oral dose of dextromethorphan, is widely used as a marker of CYP2D6 activity in adults and infants.[17,19,20] Additionally, the *N*-demethylation activity of dextromethorphan has been proposed as a means of predicting the CYP3A activity in humans.[19,21] With some limitations,[22,23] the urinary molar ratio of dextromethorphan to 3-methoxymorphinan (i.e. dextromethorphan/3-methoxymorphinan) may provide a surrogate measurement for CYP3A activity.[17,24] In addition, measurement of the endogenous free cortisol level may represent a safe, simple and non-invasive assay of the CYP3A activity. Although there is considerable debate as to which method is the most accurate for measuring the activity of CYP3A *in vivo*,[25] it has been suggested that the urinary ratio of 6β-hydroxycortisol (6β-HC) to free cortisol is a useful marker of both the induction and the inhibition of hepatic CYP3A activity.[26]

The aim of this study was to evaluate the effects of keishi-bukuryo-gan on the activity of CYP1A2, XO, NAT2, CYP2D6 and CYP3A in healthy female volunteers using caffeine and dextromethorphan as phenotyping probes, and by performing a urinary assay of 6β-HC and free cortisol.

Materials and Methods

Materials

Keishi-bukuryo-gan (TJ-25) was obtained from Tsumura & Co. (Tokyo, Japan). Seven-and-a-half grams of TJ-25 (Tsumura Keishibukuryogan extract granules) contains 1.75 g of a dried extract of the following mixed crude drugs: 3.0 g of cinnamon bark (*Cinnamomum cassia* Blume), 3.0 g of Poria Sclerotium (*Poria cocos* Wolf), 3.0 g of peony root (*Paeonia lactiflora* Pallas), 3.0 g of moutan bark (*Paeonia suffrutiosa* Andrews), 3.0 g of peach kernels (*Prunus persica* Batch) and inactive ingredients: light anhydrous silicic acid, magnesium stearate and lactose hydrate. TJ-25 has been approved for medical use. The quality variation among batches was minimized by using only Japanese pharmacopoeial quality substances, and the presence of the spots of active ingredients of the five herbal components were ascertained in every batch during the production process. The 1,7-dimethyl-uric-acid (17U), 1-methylxanthine (1X), 1-methyl-uric-acid (1U), dextromethorphan, dextrorphan, 3-methoxymorphinan, 6β-HC and free cortisol were purchased from Sigma Chemical Co. (St Louis, USA), and 5-acethyl-amino-6-formylamino-3-methyluracil (AFMU) was purchased from Welfide Co. (Osaka, Japan). All other analytical-grade reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Subjects

Thirty-one healthy female Japanese university students participated in this study. This study was approved by the Institutional Review Board of the Faculty of Life Sciences,

Kumamoto University and informed consent, including the statement regarding the privacy policy, was obtained in writing from each volunteer before entry into the study. The subjects ranged in age from 20 to 27 years (median, 22.3 years); the weight range of the subjects was 41–59 kg (mean ± SD, 48.9 ± 4.65 kg). All volunteers were judged to be healthy according to their medical histories. Each volunteer was required to be a non-smoker and not to be a user of oral contraceptives. Participants were excluded for the following reasons: allergy to keishi-bukuryo-gan, any herb, any non-herbal medicine, dextromethorphan or caffeine; a history of any infectious disease within four weeks before enrollment; use of keishi-bukuryo-gan or other herbal medicines within two weeks before enrollment; use of prescription or over-the-counter medications or alcohol within two days before enrollment; use of an investigational drug within three months; and current pregnancy or suspected pregnancy.

Study design

This was an open-label study and each subject served as her own control. The subjects were not allowed to use prescription and over-the-counter medications, or to consume any food or beverage containing xanthine (e.g. coffee, tea, colas, chocolate, etc.), fruit products or alcohol from the 48 h before the first probe drug administration test through to the end of the study. The subjects were instructed to take 3.75 g of keishi-bukuryo-gan twice daily, a standard dosage used for the treatment of menopausal symptoms, for seven days before their morning and evening meals. The phenotyping test was performed twice: on the day before (baseline) and on the 7th day (day 7) after receiving the keishi-bukuryo-gan. Compliance was assessed by a pill count and self-reporting of missed doses at the end of the study. The procedure for the phenotyping test was as follows: after emptying their bladders, the subjects received a 150-mg oral dose of caffeine (Merck, Darmstadt, Germany) and a 30-mg dose of dextromethorphan (Medicon; Shionogi Ltd, Osaka, Japan) before they went to sleep. An overnight urine sample was collected from each subject (from approximately 1100 h to 0700 h). The mean length of time for urine collection was 7.9 ± 0.68 h. Spot urine samples for each subject before the administration of caffeine and dextromethorphan on the baseline day, and on day 7 were also collected as blank controls. Thereafter, 1 ml of urine sample acidified by ascorbic acid (10 mg/ml), and a 20-ml urine sample without ascorbic acid were stored at −30°C until the high-performance liquid chromatography (HPLC) analyses of caffeine, dextromethorphan, free cortisol and their metabolites, were performed.

Analytical procedures

The urinary concentrations of three caffeine metabolites, 17U, 1U and 1X, and those of dextromethorphan, dextrorphan, 3-methoxymorphinan, 6β-HC and free cortisol were quantified by the validated HPLC analyses as reported previously.[15,17] After complete conversion of AFMU into 5-acetylamino-6-amino-3-methyluracil (AAMU) at pH 10, the AAMU was measured by an HPLC instrument with an ultraviolet detection as described previously.[15]

Statistical analysis

The activity levels of CYP1A2, XO and NAT2 were assessed using the phenotypic indices determined by the urinary molar concentrations (i.e. (AAMU+1U+1X)/17U, 1U/(1U+1X) and AAMU/(AAMU+1X+1U), respectively).[15–17] CYP2D6 activity was defined as the urinary molar ratio of dextromethorphan/dextrorphan.[17,21,23,24] The CYP3A activity was assessed by the two urinary molar ratios of dextromethorphan/3-methoxymorphinan and 6β-HC/free cortisol.[15,24] High values of the indices for CYP1A2, XO and NAT2, and of the 6β-HC/free cortisol index for CYP3A indicate high activity levels. Conversely, high values of the dextromethorphan/dextrorphan and dextromethorphan/3-methoxymorphinan indices indicate low activity levels of CYP2D6 and CYP3A, respectively. The data were reported as the geometric means ± SD. The geometric mean ratios and 90% confidence intervals (CI) were calculated for each phenotypic index as the ratio of the geometric mean of day 7 to that of baseline.[27] We concluded that there was a lack of effect of keishi-bukuryo-gan if the 90% CI was within the range of 0.80–1.25.[27] We also used the paired *t*-test to compare the log-transformed phenotypic indices between the baseline and day 7. $P < 0.05$ was considered to be statistically significant. The number of subjects was estimated to be sufficient to detect a 50% difference in the log-transformed metabolic index for CYP1A2 activity between the baseline and day 7, with a power of 0.80 (α-level of 0.05). These statistical analyses were performed with the R software program (version 2.8.1, R Foundation for Statistical Computing, Vienna, Austria).

Results

Thirty-one subjects completed the study protocol, but five were unable to collect sufficient urine samples to assess the urinary molar concentrations of free cortisol and 6β-HC. All enrolled subjects reported that they had taken all of the dispensed study medications as directed. Keishi-bukuryo-gan was well tolerated without any adverse effects during the study period.

The phenotypic indices for CYP1A2, XO and NAT2 at baseline ranged from 2.19 to 29.15 (a 13-fold difference), 0.45 to 0.87 (a 2-fold difference) and 0.10 to 0.62 (a 6-fold difference), respectively. The geometric mean CYP1A2 index significantly decreased by 16% on day 7, compared with that of the baseline ($P = 0.026$, Figure 1a, Table 2). The geometric mean ratio (90% CI) of day 7 to the baseline for the CYP1A2

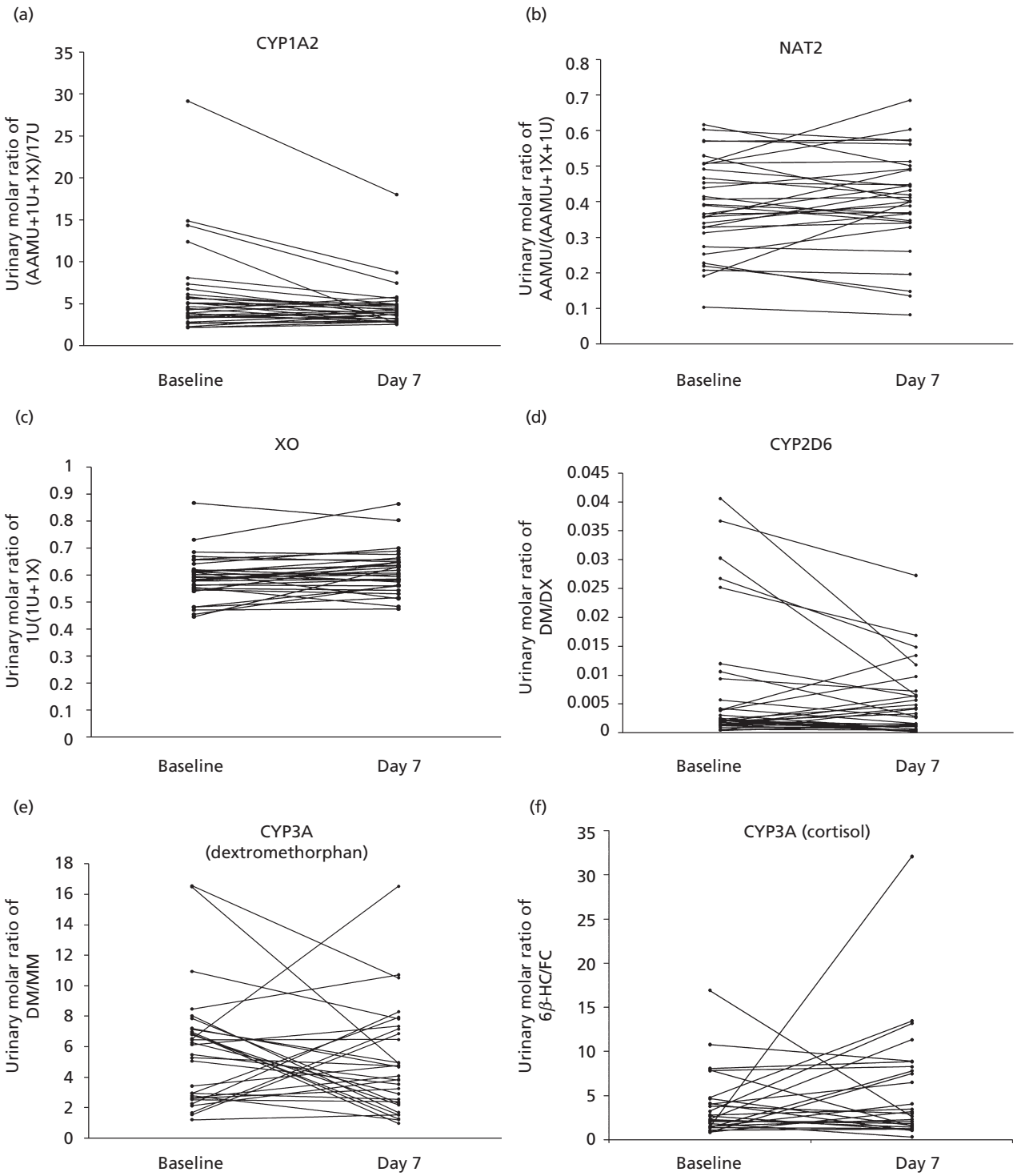

Figure 1 The individual values of the urinary molar concentration ratios of (AAMU+1U+1X)/17U for CYP1A2 (a), 1U/(1U+1X) for XO (b), AAMU/(AAMU/1X+1U) for NAT2 (c), DM/DX for CYP2D6 (d) and DM/MM for CYP3A (e) activity in 31 healthy female subjects, and of 6β-HC/FC for CYP3A (f) activity in 26 healthy female subjects, before (baseline) and on the seventh day (day 7) after repeated 7.5-g oral doses of keishi-bukuryo-gan. CYP, cytochrome P450; XO, xanthine oxidase; NAT2, *N*-acetyltransferase 2; AAMU, 5-acetylamino-6-amino-3-methyluracil; 1U, 1-methyl-uric-acid; 1X, 1-methyxanthine; 17U, 1,7-dimethyl-uric-acid; DM, dextromethorphan; DX, dextrorphan; MM, 3-methoxymorphinan; DM, dextromethorphan; DX, dextrorphan; MM, 3-methoxymorphinan; 6β-HC, 6β-hydroxycortisol; FC, free cortisol.

Table 2 The phenotyping indices for the CYP1A2, XO, NAT2, CYP2D6 and CYP3A activity on the day before (baseline) and on the seventh day (day 7) after administrating keishi-bukuryo-gan

Enzyme	Phenotypic index	Baseline[a]	Day 7[a]	Day 7/baseline ratio[b]	*P*-value[c]
CYP1A2	(AAMU+1U+1X)/17U	4.92 ± 5.45	4.11 ± 2.92	0.84 (0.73–0.95)	0.026
XO	1U/(1U+1X)	0.59 ± 0.08	0.61 ± 0.08	1.04 (1.01–1.07)	0.058
NAT2	AAMU/(AAMU+1X+1U)	0.37 ± 0.13	0.37 ± 0.14	1.01 (0.94–1.09)	0.73
CYP2D6	DM/DX	0.0032 ± 0.0114	0.0026 ± 0.0061	0.81 (0.58–1.15)	0.321
CYP3A	DM/MM	4.74 ± 3.78	3.89 ± 3.47	0.82 (0.62–1.09)	0.242
CYP3A	6β-HC/FC	2.66 ± 2.64	3.49 ± 6.78	1.31 (0.80–1.83)	0.44

17U, 1,7-dimethyl-uric-acid; 1U, 1-methyl-uric-acid; 1X, 1-methyxanthine; 6β-HC, 6β-hydroxycortisol; AAMU, 5-Acetylamino-6-amino-3-methyluracil; CYP, cytochrome P450; DM, dextromethorphan; DX, dextrorphan; FC, free cortisol; MM, 3-methoxymorphinan; NAT2, *N*-acetyltransferase 2; XO, xanthine oxidase. [a]The data are given as the geometric means ± SD. [b]The ratio of the geometric mean of day 7 to that of baseline for each phenotyping index, with 90% confidence interval in parenthesis. [c]The log-transformed phenotypic indices of the baseline and day 7-values were compared by a paired *t*-test.

activity was 0.84 (0.73–0.95). No statistically significant differences were observed in the indices for either the XO activity or the NAT2 activity between the baseline and day 7 ($P = 0.058$ and $P = 0.730$, respectively) (Figure 1b and 1c, Table 2). The ratios and 90% CIs of both the NAT2 and XO activity were included within the 80–125% bioequivalence range (Table 2).

The dextromethorphan/dextrorphan index for CYP2D6 activity at the baseline ranged from 0.0004 to 0.0406 (a 102-fold difference). The mean value of the dextromethorphan/dextrorphan index on day 7 did not differ from that of the baseline ($P = 0.321$), and the geometric mean ratio of day 7 to baseline for the CYP2D6 activity was included within the 80–125% bioequivalence range (Figure 1d, Table 2).

The dextromethorphan/3-methoxymorphinan and 6β-HC/free cortisol indices, used as surrogate markers of CYP3A activity, ranged from 1.20 to 16.56 (a 14-fold difference) and 0.85 to 16.9 (20-fold difference), respectively, at the baseline. The dextromethorphan/3-methoxymorphinan and 6β-HC/free cortisol indices on day 7 did not differ from those of the baseline ($P = 0.242$ and 0.44, Figure 1e and 1f, respectively, Table 2).

Discussion

This study demonstrated that seven-day repeated dosing of keishi-bukuryo-gan, administered to healthy female volunteers, inhibited CYP1A2 activity, but did not affect the activity of CYP2D6, CYP3A, XO or NAT2.

Many patients use complementary and alternative treatments for their menopausal symptoms.[6] In Japan, keishi-bukuryo-gan is approved for medical use in the treatment of menopausal symptoms. Therefore, we assessed the effects of keishi-bukuryo-gan on drug-metabolizing enzyme activity in healthy female volunteers, because of the obvious differences in metabolic activity between males and females.[28]

CYP1A2 metabolizes the endogenous oestrogens, 17β-estradiol (E_2) and estrone (E_1), to 2-hydroxylated metabolites, and this is the predominant metabolic pathway of these oestrogens.[29] The seven-day repeated administration of keishi-bukuryo-gan to healthy female volunteers caused a significant decrease in the activity of CYP1A2 by 16% (Figure 1a and Table 2). Therefore, we are tempted to assume that keishi-bukuryo-gan may have an impact on the disposition of endogenous and/or exogenous oestrogens. A previous study reported that there was no significant change in the endogenous serum estradiol levels after long-term treatment with keishi-bukuryo-gan in six postmenopausal female patients.[7] However, there are no available data on whether there is an improved efficacy and/or adverse events in relation to the interaction between keishi-bukuryo-gan and exogenous oestrogens, such as hormone replacement therapy. Our results may suggest that the disposition, efficacy and safety of exogenous oestrogens should be carefully monitored in patients treated with keishi-bukuryo-gan. Further in-vivo studies in humans are therefore necessary to assess whether keishi-bukuryo-gan may interact with exogenous oestrogens to a clinically significant extent.

Among the main ingredients of keishi-bukuryo-gan, the *O*-methoxycinnamaldehyde present in cinnamon bark has been reported to inhibit CYP1A2 activity in rat liver microsomes[9] (Table 1). We previously assessed the effects of other Kampo medicines, such as sho-saiko-to, bakumondo-to and sho-seiryu-to, on the CYP1A2 activity in healthy volunteers using the same caffeine test.[15–17] The components of sho-saiko-to and bakumondo-to are completely different to those in keishi-bukuryo-gan,[15,16] whereas sho-seiryu-to also contains cinnamon bark.[17] However, sho-seiryu-to did not influence the CYP1A2 activity in our previous study.[17] One possible explanation is that sho-seiryu-to, but not keishi-bukuryo-gan, contains glycyrrhizin, which is known to induce CYP1A2 activity in rat and murine

livers.[30] Further studies are required to investigate which ingredient(s) are responsible for the potential inhibitory effects of keishi-bukuryo-gan on CYP1A2 that we observed in this present study.

CYP3A is involved in the metabolism of many endogenous and exogenous compounds, including oestrogens.[29] Among the components of keishi-bukuryo-gan, Poria Sclerotium has been demonstrated to induce the expression of CYP3A4 in HepG2 cells,[13] whereas some ingredients of cinnamon bark have been reported to inhibit the activity of CYP3A *in vitro*[10,11] (Table 1). In this study, we demonstrated that the 6β-HC/free cortisol and dextromethorphan/3-methoxymorphinan indices did not differ between baseline and day 7.

Several assessments of the CYP3A phenotype have been carried out *in vivo*.[20,23–26,31] Of these markers, the most widely used probe drug is midazolam; however, repeated blood sampling is required to measure the clearance of this drug.[31] The urinary excretion ratio of endogenous 6β-HC/free cortisol may therefore represent a safe, simple and non-invasive assay of CYP3A activity.[26] In addition, correlation, inhibition and induction studies have suggested that the dextromethorphan/3-methoxymorphinan index reflects the CYP3A activity.[17,24] However, a previous study demonstrated that the values of the dextromethorphan/3-methoxymorphinan index depended on those of the dextromethorphan/dextrorphan index for CYP2D6.[23] We also reported that the dextromethorphan/3-methoxymorphinan index was strongly influenced by the *CYP2D6* genotypes.[17] Nevertheless, the findings of this study demonstrated that keishi-bukuryo-gan does not influence the 6β-HC/free cortisol and dextromethorphan/3-methoxymorphinan indices, thus suggesting that it has no effect on the CYP3A activity in healthy females.

The urinary molar ratio of dextromethorphan to dextrorphan is widely used as a marker of in-vivo CYP2D6 activity.[19,20] In this study, we observed no significant difference in the log-transformed dextromethorphan/dextrorphan index between blood samples obtained before and after receiving keishi-bukuryo-gan (Figure 1d, Table 2). Therefore, keishi-bukuryo-gan also does not seem to affect the CYP2D6 activity in healthy females.

Several components of keishi-bukuryo-gan can affect the XO and NAT2 activity *in vitro* (Table 1). Pentagalloylglucose was previously reported to inhibit cow's milk-derived XO activity, and its inhibitory effect was comparable with that of the potent XO inhibitor allopurinol.[12] Paeonol was shown to increase the NAT2 activity in human colon tumour cells.[14] However, we observed no significant differences in the log-transformed phenotypic indices for XO and NAT2 activity before and after dosing with keishi-bukuryo-gan, and the 90% CI ranges of the geometric mean ratios for XO and NAT2 were included within the bioequivalence range defined by the US Food and Drug Administration.[27]

Conclusions

Our findings may suggest that the disposition, efficacy and safety of exogenous oestrogens should be carefully monitored in patients treated with keishi-bukuryo-gan. Since our results were obtained from healthy and premenopausal female volunteers, further in-vivo studies in humans are necessary to assess whether keishi-bukuryo-gan would interact with exogenous oestrogens to a clinically significant extent. Nevertheless, our findings suggest that keishi-bukuryo-gan is unlikely to participate in herb–drug interactions involving medications predominantly metabolized by CYP2D6, CYP3A, XO and NAT2.

Declarations

Conflict of interest

Prof. Nakagawa has a research grant from Tsumura & Co. The authors have no other funding, financial relationships, or conflicts of interest to disclose.

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