Jpn. J. Pharmacol. **89**, 267 – 273 (2002)

Effects of *Wu-chu-yu-tang* and Its Component Herbs on Drug-Metabolizing Enzymes

Yune-Fang Ueng[1,*], Ming-Jaw Don[1], Hsiao-Chi Peng[1], Shu-Yun Wang[1], Jong-Jing Wang[2] and Chieh-Fu Chen[1,2]

[1]*National Research Institute of Chinese Medicine, 155-1, Li-Nong Street, Sec. 2, Taipei 112, Taiwan*
[2]*Institute of Bio-Pharmaceutical Science, National Yang-Ming University, 155, Li-Nong Street, Sec. 2, Taipei 112, Taiwan*

Received January 25, 2002 Accepted April 3, 2002

ABSTRACT—The compound herbal medicine *Wu-chu-yu-tang* is used for the treatment of migraine and vomiting accompanying a cold. To assess the interactions of herb and drug metabolism, effects of *Wu-chu-yu-tang* on hepatic and renal cytochrome P450 (CYP), UDP-glucuronosyl transferase (UGT) and glutathione *S*-transferase (GST) were studied in C57BL/6J mice. Treatment of mice with 5 g/kg per day *Wu-chu-yu-tang* for 3 days caused 2.5-fold and 2.9-fold increases of liver microsomal 7-ethoxyresorufin *O*-deethylation (EROD) and 7-methoxyresorufin *O*-demethylation activities, respectively. However, CYP activities toward 7-ethoxycoumarin, benzphetamine, *N*-nitrosodimethylamine, erythromycin and nifedipine, and conjugation activities of UGT and GST were not affected. In kidney, *Wu-chu-yu-tang*-treatment had no effects on Cyp, UGT and GST activities. Among the four component herbs of *Wu-chu-yu-tang*, only *Evodiae Fructus* (*Wu-chu-yu*) extract increased EROD activity and CYP1a2 protein level. In *E. Fructus*, rutaecarpine, evodiamine and dehydroevodiamine are the main active alkaloids. At the doses corresponding to their contents in *Wu-chu-yu-tang*, rutaecarpine-treatment increased hepatic EROD activity, whereas evodiamine and dehydroevodiamine had no effects. These results demonstrated that ingestion of *Wu-chu-yu-tang* elevated mouse hepatic Cyp1a2 activity and protein level. *E. Fructus* and rutaecarpine contributed at least in part to the CYP1a2 induction by *Wu-chu-yu-tang*. Patients should be cautioned about the drug interaction of *Wu-chu-yu-tang* and CYP1A2 substrates.

Keywords: *Wu-chu-yu-tang*, *Evodiae Fructus*, Rutaecarpine, Drug-metabolizing enzyme, Liver

Due to the increase in the use of herbal remedies, physicians and pharmacists are very much concerned about the toxicity and drug interactions when using herbal medicines. Several medicinal herbs such as *Ginkgo biloba* and *St John's wort* have been reported to show interactions with pharmaceutical drugs (1, 2). Modulation of drug-metabolizing enzymes is one of the main causes of drug interactions (3, 4). Oxidation and conjugation are two main reactions involved in drug metabolism. Cytochrome P450 (CYP)-dependent monooxygenases are the primary oxidation enzyme systems involved in the detoxication and bioactivation of a number of drugs and environmental pollutants (5). The oxidations catalyzed by the monooxygenase system require a CYP enzyme, NADPH-CYP reductase and phospholipids. The CYP family comprises a group of enzymes with broad substrate specificity. This substrate specificity leads to the herb-induced drug interactions with selective CYP substrates. For conjugation reactions, UDP-glucuronosyl transferase (UGT) and glutathione *S*-transferase (GST) are important enzymes, which catalyze the glucuronide and glutathione conjugates formation, respectively. Changes of conjugation activities can also affect the detoxication and excretion of drugs (6).

Wu-chu-yu-tang (*Goshuyu-to* in Japanese *Kampo* medicine) is a traditional Chinese prescription for the treatment of migraine and vomiting accompanying a cold (7). *Wu-chu-yu-tang* contains *Evodiae Fructus* (*Wu-chu-yu*, *Evodia rutaecarpa*), *Ginseng Radix* (ginseng, *Panax ginseng*), *Zingiber Rhizoma* (ginger, *Zingiber officinale*) and *Zizyphi Fructus* (*Tai-Geui*, *Ziziphus jujuba*). The powdered extract of *Wu-chu-yu-tang* is commercially available and has been commonly used in Taiwan and Japan. Our previous report demonstrated that rutaecarpine, an alkaloid isolated from *E. rutaecarpa*, had a strong inductive effect on mouse hepatic CYP1a1/1a2 (8). However, the experimental studies of drug interactions by *Wu-chu-yu-tang* have not been reported. To assess the drug interaction of *E. rutaecarpa-*

*Corresponding author. FAX: +886-2-28264266
E-mail: ueng@cma23.nricm.edu.tw

containing compound medicine, effects of *Wu-chu-yu-tang* on CYP, UGT and GST were studied in mouse liver and kidney. The roles of component herbs and alkaloids in the modulation by *Wu-chu-yu-tang* are identified.

MATERIALS AND METHODS

The powdered extracts of compound medicine *Wu-chu-yu-tang* and its component herbs were purchased from Sun Ten Pharmaceutical Co. (Taipei, Taiwan). Nine grams of *Wu-chu-yu-tang* contains 4 g starch and 5 g decoction concentrate prepared from 7.5 g *E. Fructus*, 9.0 g *Z. Rhizoma*, 4.5 g *G. Radix* and 6.0 g *Z. Fructus*. Rutaecarpine was synthesized following the method of Bergman and Bergman (9). Evodiamine and dehydroevodiamine were isolated from *E. Fructus* (10). Benzo(a)pyrene, cytochrome *c*, NADPH, 7-ethoxyresorufin, 7-ethoxycoumarin and nifedipine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 3-Hydroxybenzo(a)pyrene was purchased from NCI Chemical Carcinogen Reference Standard Repository (Kansas city, MO, USA). Acrylamide and Bis-acrylamide were purchased from BioRad Pacific Ltd. (Kowloon, Hong Kong). Horseradish peroxidase conjugated rabbit anti-mouse IgG was purchased from Pierce Chemical Co. (Rockford, IL, USA).

Effects of Wu-chu-yu-tang and its component herbs and alkaloids on drug-metabolizing enzymes

Animal treatment and subcellular fraction preparation: Male C57BL/6J mice (5-week-old, weighing 13 – 16 g) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Before experimentation, mice were allowed a one-week acclimation period at the animal quarters with air conditioning (25 ± 1°C) and an automatically controlled photoperiod of 12 h light daily. All experimental protocols involving animals were reviewed and approved by the Institutional Animal Experimentation Committee of National Research Institute of Chinese Medicine. Mice were given water and laboratory rodent chow (#5P14; PMI Feeds Inc., Richmond, IN, USA) ad libitum throughout the entire experiment. The concentrates of *Wu-chu-yu-tang*, *E. Fructus*, *G. Radix*, *Z. Rhizoma* and *Z. Fructus* were suspended in water. Mice were treated with concentrates by gastrogavage for three days at the dosages as described in the figure legends and tables. The control group received the same volume of water per kg body weight. For induction of CYP1a1 and CYP1a2, mice were treated with a single injection of 3-methylcholanthrene at 80 mg/kg intraperitoneally and liver microsomes were prepared 48 h after the injection. Microsomes and cytosols isolated from liver and kidney were prepared by differential centrifugation (11).

Enzyme assays: CYP and cytochrome b_5 contents were determined following the method of Omura and Sato (12). NADPH-CYP reductase activity was determined using cytochrome *c* as a substrate (13). In the benzo(a)pyrene hydroxylation assay, formation of phenolic benzo(a)pyrene was determined using 3-hydroxybenzo(a)pyrene as a standard (14). The *O*-dealkylations of 7-ethoxyresorufin and 7-methoxyresorufin were determined by measuring fluorescence of resorufin (15). 7-Ethoxycoumarin *O*-deethylation was determined by measuring the fluorescence of hydroxycoumarin (16). *N*-Demethylations of benzphetamine and *N*-nitrosodimethylamine were determined by measuring the formation of formaldehyde using Nash's reagent (17). Nifedipine oxidation was determined following the method of Guengerich et al. (18). Microsomal UGT activity was determined using *p*-nitrophenol as a substrate (19). Cytosolic GST activity was determined using 1-chloro-2,4-dinitrobenzene as a substrate in the presence of glutathione (20). Microsomal and cytosolic protein concentrations were determined by the method of Lowry et al. (21).

Immunoblot analysis: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the discontinuous system of Laemmli (22). Microsomal and cytosolic proteins were electrophoresed on a 10% and 12% (w/v) polyacrylamide gel, respectively. Electrophoresis was carried out at 4°C and at 15 mamp/gel during stacking and 30 mamp/gel during separation. Following electrophoresis, microsomal proteins were transferred from the slab gel to a nitrocellulose membrane using the method of Towbin et al. (23). Immunodetection of CYPs in liver microsomes was performed using mouse monoclonal antibodies against rat CYP1A1 (MAb 1-7-1) (24). Monoclonal antibodies against rat CYPs were obtained from Dr. Sang-Shin Park (Seoul National University, Seoul, Korea). Immunoreactive proteins were detected by rabbit IgGs against mouse IgGs conjugated with horseradish peroxidase and immunostained using a chemiluminescence detection kit from Amersham (Buckinghamshire, UK). The protein band density was analyzed by densitometry using ImageMaster (Pharmacia Biotech Ltd., Uppsala, Sweden).

LC-MS analysis of the alkaloids of Wu-chu-yu-tang: One milligram of *Wu-chu-yu-tang* was extracted with 2 ml chloroform and the supernatant was collected after centrifugation at 13,500 rpm for 10 min. The supernatant was dried under N_2 gas at room temperature and re-dissolved in acetonitrile. This extract solution was injected into a C18 column (5 μm, 4.6 × 250 mm, Cosmosil 5C18-MS-II; Nacalai Tesque, Kyoto). The alkaloids were separated by gradient using A: 15% acetonitrile, 0.05% formic acid and B: 85% acetonitrile, 0.05% formic acid at a flow rate of 0.5 ml/min. The linear gradient was as follows: 0 – 7 min, 20% to 50% B; 7 – 27 min, 50% to 80% B; 27 – 37 min, 80% to 100% B. Alkaloids were detected by mass spectrometer in selected ion monitoring mode (spray voltage:

4.5 kV, capillary voltage: 10 V, capillary temperature: 270°C, sheath gas, 80 units, auxiliary gas: 20 units; LCQ ion trap mass spectrometer; Finnigan Co., Ltd., San Jose, CA, USA). Amounts of alkaloids were determined using the peak area of the $[M+H]^+$ ion for rutaecarpine (m/z=288), evodiamine (m/z=304) and dehydroevodiamine (m/z=302).

Statistical analyses

The statistical significance of difference between control and herbal concentrate-treated groups was evaluated by Student's *t*-test. The differences between >2 sets of data (control and groups treated with various doses of *Wu-chu-yu-tang*) were analyzed by one-way ANOVA followed by Dunnett's test for multiple comparisons. A P value <0.05 was considered as statistically significant.

RESULTS

Dose-response of effect of Wu-chu-yu-tang on EROD activity in mouse liver

To examine the dose-dependent effect of *Wu-chu-yu-tang* on Cyp-catalyzed 7-ethoxyresorufin *O*-deethylation (EROD) activity, mice were treated with increasing dosages of *Wu-chu-yu-tang* for three days. Treatment of mice with 1, 2 and 5 g/kg per day *Wu-chu-yu-tang* suspension caused 75%, 2.1-fold and 2.4-fold increases of EROD activity in mouse liver (Fig. 1). Thus, mice were treated with 5 g/kg per day *Wu-chu-yu-tang* for three days in the following studies.

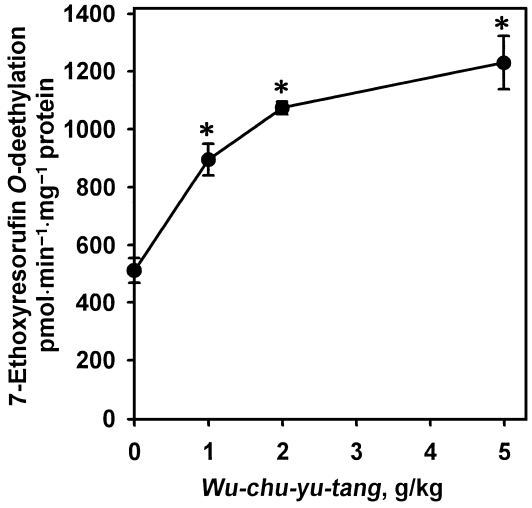

Fig. 1. Dose-response of effect of *Wu-chu-yu-tang* on 7-ethoxyresorufin *O*-deethylation activity in mouse liver. Mice were treated with *Wu-chu-yu-tang* for three days at the indicated dose. Microsomes were prepared 20 h after the final treatment and enzyme assays were performed within one week. Results represent the means ± S.E.M. of four mice. Asterisks indicate values significantly different from the control values as analyzed by one-way ANOVA followed by Dunnett's test, P<0.05.

Effect of Wu-chu-yu-tang on CYP, UGT and GST activities in mouse liver and kidney

Wu-chu-yu-tang-treatment had no effect on mouse body and tissue weights (data not shown). *Wu-chu-yu-tang*-treated mice had liver microsomal CYP content similar to that of control mice (Table 1). This treatment caused a 22% increase of cytochrome b_5 content without affecting NADPH-CYP reductase activity. *Wu-chu-yu-tang*-treatment resulted in 2.5-fold and 2.9-fold increases of hepatic EROD and 7-methoxyresorufin *O*-deethylation (MROD) activities, respectively (Table 2). In contrast, *Wu-chu-yu-tang* had no effects on oxidations of 7-ethoxycoumarin, benzphetamine, *N*-nitrosodimethylamine, erythromycin and nifedipine activities. Liver microsomal UGT and cytosolic GST activities were not affected by this treatment. In kidney, *Wu-chu-yu-tang* had no effects on benzo(a)pyrene hydroxylation, 7-ethoxycoumarin *O*-deethylation, UGT and GST activities (Table 3). Renal EROD and MROD activities were not detectable in both control and *Wu-chu-yu-tang*-treated groups.

Effect of Wu-chu-yu-tang on the level of CYP1A-immunoreactive protein

Immunoblot analysis of microsomal proteins revealed that *Wu-chu-yu-tang*-treatment increased the level of a CYP1a2-immunoreactive protein. The electrophoretic mobility of *Wu-chu-yu-tang*-induced protein was similar to that of CYP1a2 present in control mice and 3-methylcholanthrene-treated mice (Fig. 2A). *Wu-chu-yu-tang*-treatment caused a 2.1-fold increase of the intensity of this protein band as analyzed by densitometry.

Effect of the component herbs of Wu-chu-yu-tang on hepatic EROD activity and the level of CYP1A-immunoreactive protein in mouse liver

To illustrate the roles of component herbs in the induction of CYP1A by *Wu-chu-yu-tang*, mice were treated with

Table 1. Effect of *Wu-chu-yu-tang* on components of monooxygenase system in mouse liver

Assay	Control	*Wu-chu-yu-tang*
Cytochrome P450 (nmol/mg protein)	0.66 ± 0.04	0.66 ± 0.09
Cytochrome b_5 (nmol/mg protein)	0.36 ± 0.01	0.44 ± 0.01*
NADPH-Cytochrome P450 reductase (μmol·min^{-1}·mg^{-1} protein)	0.34 ± 0.01	0.37 ± 0.01

Microsomes of individual mouse liver were prepared and enzyme activities were determined as described in the section of Materials and Methods. Results represent the means ± S.E.M. of six mice. Asterisk indicates a value significantly different from the control value, P<0.05.

Table 2. Effects of *Wu-chu-yu-tang* on CYP, UDP-glucuronosyl transferase and glutathione *S*-transferase activities in mouse liver

Assay		Control	*Wu-chu-yu-tang*
Benzo(a)pyrene hydroxylation	(nmol·min^{-1}·mg^{-1} protein)	0.60 ± 0.07	0.80 ± 0.02
7-Ethoxyresorufin *O*-deethylation	(nmol·min^{-1}·mg^{-1} protein)	0.50 ± 0.03	1.27 ± 0.06*
7-Methoxyresorufin *O*-demethylation	(nmol·min^{-1}·mg^{-1} protein)	1.09 ± 0.10	3.12 ± 0.24*
7-Ethoxycoumarin *O*-deethylation	(nmol·min^{-1}·mg^{-1} protein)	2.75 ± 0.21	3.50 ± 0.30
Benzphetamine *N*-demethylation	(nmol·min^{-1}·mg^{-1} protein)	5.56 ± 0.24	6.62 ± 0.39
N-Nitrosodimethylamine *N*-demethylation	(nmol·min^{-1}·mg^{-1} protein)	2.77 ± 0.26	3.17 ± 0.31
Erythromycin *N*-demethylation	(nmol·min^{-1}·mg^{-1} protein)	3.82 ± 0.67	3.69 ± 0.30
Nifedipine oxidation	(nmol·min^{-1}·mg^{-1} protein)	0.58 ± 0.06	0.55 ± 0.03
UDP-Glucuronosyl transferase (*p*-nitrophenol glucuronidation)	(nmol·min^{-1}·mg^{-1} protein)	26.5 ± 6.5	19.4 ± 3.9
Glutathione *S*-transferase (1-chloro-2,4-dinitrobenzene glutathione conjugation)	(μmol·min^{-1}·mg^{-1} protein)	5.21 ± 0.71	5.42 ± 0.33

Mice were treated with 5 g/kg per day *Wu-chu-yu-tang* for three days. Control group received same volumes of water. Results represent the means ± S.E.M. of six mice. Asterisks indicate values significantly different from the control values, $P<0.05$.

Table 3. Effect of *Wu-chu-yu-tang* on CYP activities and conjugation enzyme activities in mouse kidney

Assay		Control	*Wu-chu-yu-tang*
Benzo(a)pyrene hydroxylation	(pmol·min^{-1}·mg^{-1} protein)	23.2 ± 1.4	32.1 ± 3.9
7-Ethoxycoumarin *O*-deethylation	(pmol·min^{-1}·mg^{-1} protein)	73.7 ± 14.0	75.6 ± 9.0
UDP-Glucuronosyl transferase (*p*-nitrophenol glucuronidation)	(nmol·min^{-1}·mg^{-1} protein)	5.4 ± 1.3	8.3 ± 3.9
Glutathione *S*-transferase (1-chloro-2,4-dinitrobenzene glutathione conjugation)	(μmol·min^{-1}·mg^{-1} protein)	1.3 ± 0.1	1.1 ± 0.3

Microsomes of individual mouse liver were prepared and enzyme activities were determined as described in the section of Materials and Methods. Results represent the means ± S.E.M. of six mice.

component herbal extracts at the dosage comparable to their contents in *Wu-chu-yu-tang*. The contents of individual herbal concentrates in *Wu-chu-yu-tang* were estimated based on same concentrate yield prepared from individual herbal decoction. Therefore, one gram of *Wu-chu-yu-tang* contains 0.15 g *E. Fructus*, 0.19 g *G. Radix*, 0.93 g *Z. Rhizoma*, and 0.12 g *Z. Fructus* extracts. To make it comparable to 5 g/kg *Wu-chu-yu-tang* treatment, mice were treated with 0.75 g/kg *E. Fructus*, 0.95 g/kg *G. Radix*, 0.47 g/kg *Z. Rhizoma*, or 0.60 g/kg *Z. Fructus*. The powdered concentrate of *E. Fructus* caused a twofold increase of hepatic EROD activity, whereas the other component herbal concentrates had no effect (Table 4). Immunoblot analysis of microsomal proteins showed that *E. Fructus* caused a 90% increase of the intensity of a CYP1a2-immunoreactive protein band (Fig. 2B).

Effects of quinazolinocarboline alkaloids of E. Fructus on hepatic EROD activity

To reveal the roles of rutaecarpine and its structurally related alkaloids in CYP1A induction by *Wu-chu-yu-tang*, contents of alkaloids in *Wu-chu-yu-tang* were determined by LC-MS (Fig. 3). The results of quantitative LC-MS analyses showed that there were 0.69 mg rutaecarpine, 0.38 mg evodiamine, and 0.60 mg dehydroevodiamine in the chloroform extract of one gram *Wu-chu-yu-tang*, respectively. Thus, treatments with 3.5 mg/kg rutaecarpine, 1.9 mg/kg evodiamine, 3.0 mg/kg dehydroevodiamine, and a mixture of three alkaloids were equivalent to treatment with 5 g/kg *Wu-chu-yu-tang* in term of amounts of alkaloids of *Wu-chu-yu-tang* administrated to mice. Rutaecarpine-treatment and the alkaloid mixture-treatment resulted in 42% and 54% increases of EROD activity, respectively (Table 5). However, evodimaine and dehydroevodiamine had no effect on EROD activity.

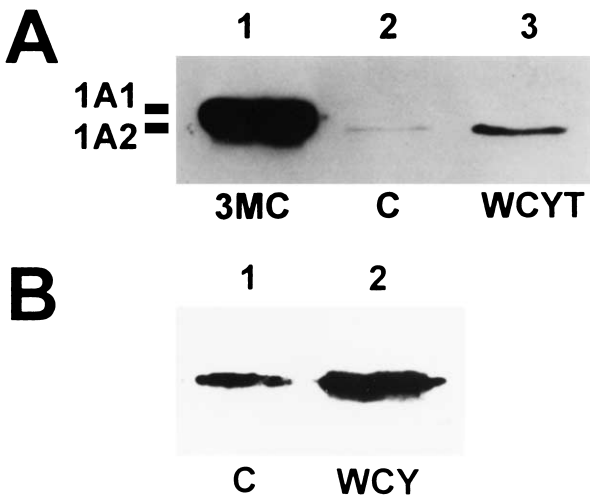

Fig. 2. Immunoblot analysis of microsomal cytochrome P450 1A in mouse liver. Panel A: Lane 1 contained 0.5 μg microsomal proteins from 3-methylcholanthrene (3MC)-treated mice. Lanes 2 and 3 contained microsomal proteins from control (C) and *Wu-chu-yu-tang* (WCYT)-treated mice. Panel B: Lanes 1 and 2 contained microsomal proteins from control (C) and *Evodiae Fructus* (WCY)-treated mice. Twenty five micrograms microsomal proteins were loaded for immunoreaction with mouse anti-rat CYP1A1 (MAb 1-7-1). Electrophoresis and immunodetection were carried out as described in the section of Materials and Methods.

Table 4. Effects of *Evodiae Fructus*, *Ginseng Radix*, *Zingiber Rhizoma* and *Zizyphi Fructus* on 7-ethoxyresorufin *O*-deethylation activity in mouse liver

Treatment	7-Ethoxyresorufin *O*-deethylation ($nmol \cdot min^{-1} \cdot mg^{-1}$ protein)
Control (n=6)	0.50 ± 0.05
Evodiae Fructus (n=5)	1.03 ± 0.12*
Ginseng Radix (n=6)	0.52 ± 0.06
Zingiber Rhizoma (n=6)	0.45 ± 0.07
Zizyphi Fructus (n=6)	0.42 ± 0.07

Mice were treated with 0.77 g/kg per day *Evodiae Fructus*, 0.47 g /kg per day *Ginseng Radix*, 0.93 g/kg per day *Zingiber Rhizoma*, and 0.62 g/kg per day *Zizyphi Fructus* for three days. Results represent the means ± S.E.M. and the number of mice in each group was shown in parentheses. Astrerisk indicates a value significantly different from the control value, $P<0.05$.

DISCUSSION

Similar to rodent CYP1A, human CYP1A can be induced by polycyclic aromatic hydrocarbons and some flavones (25). In the present report, our results showed that oral treatment of mice with *Wu-chu-yu-tang* for three days caused a significant increase of hepatic CYP1a-catalyzed EROD activity in a dose-dependent manner (Fig. 1). The dosage of powdered concentrate of *Wu-chu-yu-tang* used in human is 9 g t.i.d. Human daily dose in a 60 kg person was 45% of the minimal inducing dose (1 g/kg) used in the present mouse study. The time period required for medical treatment of humans with compound herbal prescription is generally longer than one week. Although the direct extrapolation from mouse to human is difficult, our results suggested that ingestion of *Wu-chu-yu-tang* might induce human hepatic CYP1A.

Wu-chu-yu-tang-treatment at 5 g/kg caused a significant increase of cytochrome b_5 content in mouse liver. The electron transfer protein, cytochrome b_5 is involved in the catabolism and metabolism of many endogenous and exogenous substances. The involvement of cytochrome b_5 has been reported to be important in the stimulation and inhibition of CYP activities (26). However, the implication of the induction of cytochrome b_5 in the effects of *Wu-chu-yu-*

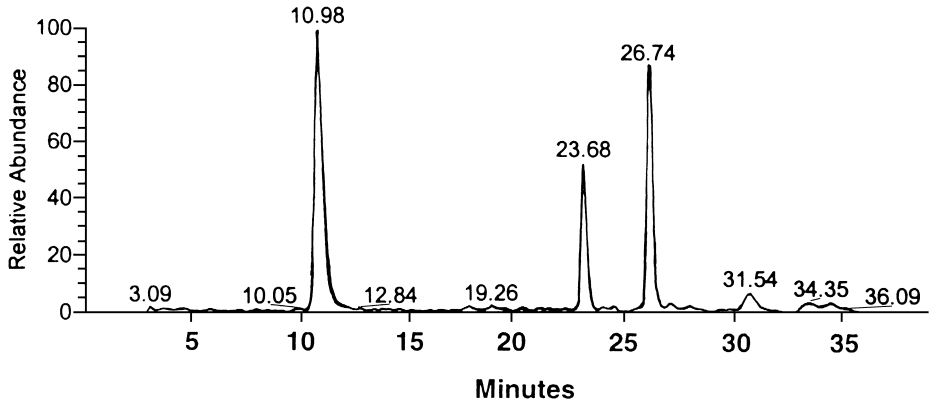

Fig. 3. The selective ion monitoring chromatogram of LC-MS analysis of the chloroform extract of *Wu-chu-yu-tang*. Results represent the relative abundance of peak height to the most abundant peak fraction. After chromatography, alkaloids in the fractions were identified by mass spectra. The fractions appeared at 11, 24 and 27 min from HPLC were dehydroevodiamine, evodiamine and rutaecarpine, respectively.

Table 5. Effects of rutaecarpine and related alkaloids on 7-ethoxyresorufin *O*-deethylation activity in mouse liver

Alkaloid	7-Ethoxyresorufin *O*-deethylation ($nmol \cdot min^{-1} \cdot mg^{-1}$ protein)
Control (n=6)	0.50 ± 0.02
Rutaecarpine (n=6)	0.71 ± 0.04*
Evodiamine (n=4)	0.54 ± 0.04
Dehydroevodiamine (n=4)	0.51 ± 0.07
Mixture of rutaecarpine, evodiamine and dehydroevodiamine (n=6)	0.77 ± 0.09*

Mice were treated with 3.5 mg/kg per day rutaecarpine, 1.9 mg/kg per day evodiamine, or 3.0 mg/kg per day dehydroevodiamine for three days. In the mixture-treated group, mice were treated with the mixture of rutaecarpine, evodiamine, and dehydroevodiamine with the dose corresponding to the dose of individual alkaloid as above. Results represent the mean ± S.E.M. The number of mice in each group is shown in parentheses. Asterisks indicate values significantly different from the control values, $P<0.05$.

tang was not clear in the present study. *Wu-chu-yu-tang*-treatment increased hepatic CYP1A-catalyzed EROD and MROD activities. However, *Wu-chu-yu-tang* had no effect on CYP2B-catalyzed benzphetamine *N*-demethylation, CYP2E1-catalyzed *N*-nitrosodimethylamine *N*-demethylation, and CYP3A-catalyzed nifedipine oxidation and erythromycin *N*-demethylation activities. (Table 2 and Fig. 2A). This result demonstrated that *Wu-chu-yu-tang* caused a selective induction of CYP1a. Immunoblot analysis of microsomal proteins showed that *Wu-chu-yu-tang* and *E. Fructus* induced a CYP1a2-immunoreactive protein. These results indicated that the induction by *Wu-chu-yu-tang* and *E. Fructus* occurred at the protein level, not from direct interaction between the herbal materials and CYP.

Among the four component herbs in *Wu-chu-yu-tang*, *E. Fructus* was the only herb that showed induction of hepatic EROD activity and the level of CYP1a2 protein (Table 3 and Fig. 2B). The induction fold of EROD activity by *E. Fructus* concentrate was similar to the induction by *Wu-chu-yu-tang*. *E. Fructus* played the main role in the induction of CYP1a2 by *Wu-chu-yu-tang*. Our previous report showed that CYP1a1 and CYP1a2 were strongly induced by rutaecarpine, the main quinazolinocarboline alkaloid in *E. Fructus*. (8). Our present result showed that treatment of rutaecarpine at the dose of 3.5 mg/kg, which was corresponded to 5 g/kg *Wu-chu-yu-tang* significantly increased hepatic EROD activity (Table 5). Treatment of mice with the mixture of rutaecarpine, evodiamine, and dehydroevodiamine increased hepatic EROD activity with the induction fold similar to the treatment of rutaecarpine only. However, the induction level was slightly less than that of *Wu-chu-yu-tang* (Tables 2 and 5). Although we can not exclude the contribution of other constituents present in *Wu-chu-yu-tang*, rutaecarpine may play a major role in the induction of CYP1a2 by *Wu-chu-yu-tang*. Our results suggest that *Wu-chu-yu-tang* is likely to interact with drugs that are mainly metabolized by CYP1A2. Thus, *Wu-chu-yu-tang* should be used with caution by patients taking drugs metabolized by CYP1A2 such as theophylline.

Acknowledgments

We thank Mr. In-Chen Chen for technical assistance for LC-MS analysis of alkaloids. This work was supported by National Research Institute of Chinese Medicine and grant NSC89-2320-B077-010 from the National Science Council, Executive Yuan, Taiwan.

REFERENCES

1 Fugh-Berman A: Herb-drug interactions. Lancet **355**, 134 – 138 (2000)
2 Liu GT: Effects of some compounds isolated from Chinese medicinal herbs on hepatic microsomal cytochrome P-450 and their potential biological consequences. Drug Metab Rev **23**, 439 – 465 (1991)
3 Guengerich FP: Role of cytochrome P450 enzymes in drug-drug interactions. Adv Pharmacol **43**, 7 – 35 (1997)
4 Lin JH and Lu AYH: Inhibition and induction of cytochrome P450 and the clinical implications. Clin Pharmacokinet **35**, 361 – 390 (1998)
5 Guengerich FP: Catalytic selectivity of human cytochrome P450 enzymes: relevance to drug metabolism and toxicity. Toxicol Lett **70**, 133 – 138 (1994)
6 de Wildt SN, Kearns GL, Leeder JS and van den Anker JN: Glucuronidation in humans. Pharmacogenetics and developmental aspects. Clin Pharmacokinetics **36**, 439 – 452 (1999)
7 Kano Y, Zong Q and Komatsu K: Pharmacological properties of Galenical preparation. XIV. Body temperature retaining effect of the Chinese traditional medicine, "*Goshuyu-to*" and component crude drugs. Chem Pharm Bull (Tokyo) **39**, 690 – 692 (1991)
8 Ueng YF, Wang JJ, Lin LC, Park SS and Chen CF: Induction of cytochrome P450-dependent monooxygenase in mouse liver and kidney by rutaecarpine, an alkaloid of the herbal drug *Evodia rutaecarpa*. Life Sci **70**, 207 – 217 (2001)
9 Bergman J and Bergman S: Studies of rutaecarpine and related quinazolinocarboline alkaloids. J Org Chem **50**, 1246 – 1255 (1985)
10 Lin LC, Chou CJ, Chen KT and Chen CF: Flavonoids from *Evodiae fructus*. J Chinese Med **2**, 94 – 100 (1991)
11 Alvares AP and Mannering GJ: Two-substrate kinetics of drug-metabolizing enzyme systems of hepatic microsomes. Mol Pharmacol **6**, 206 – 211 (1970)
12 Omura T and Sato R: The carbon monoxide-binding pigment of liver microsomes. l. Evidence for its hemeprotein nature. J Biol Chem **239**, 2370 – 2379 (1964)
13 Phillips AH and Langdon RG: Hepatic triphosphopyridine nucleotide-cytochrome *c* reductase: isolation, characterization, and kinetic studies. J Biol Chem **237**, 2652 – 2660 (1962)
14 Nebert DW and Gelboin HV: Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. l. Assembly and properties of induced enzyme. J Biol Chem **243**, 6242 – 6249 (1968)
15 Pohl RJ and Fouts JR: A rapid method for assaying the metabo-

lism of 7-ethoxyresorufin by microsomal subcellular fractions. Anal Biochem **107**, 150 – 155 (1980)

16 Greenlee WF and Poland A: An improved assay of 7-ethoxycoumarin deethylase activity: induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J Pharmacol Exp Ther **205**, 596 – 605 (1978)

17 Nash T: The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem J **55**, 416 – 421 (1953)

18 Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T and Waxman DV: Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. J Biol Chem **261**, 5051 – 5060 (1986)

19 Bock KW, Burchell B, Ditton GJ, Hanninen O, Mulder GJ, Owens IS, Siest G and Tephyl TR: UDP-Glucuronosyl transferase activities: guidelines for consistent interim terminology and assay condition. Biochem Pharmacol **32**, 953 – 955 (1983)

20 Habig WH, Pabst MJ and Jascoby WB: Glutathione *S*-transferase: the first enzymatic step in mercapturic acid formation. J Biol Chem **249**, 7130 – 7139 (1974)

21 Lowry OH, Roseborough NJ, Farr AL and Randall RL: Protein measurement with the Folin phenol reagent. J Biol Chem **193**, 265 – 275 (1951)

22 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (Lond) **227**, 680 – 685 (1970)

23 Towbin H, Staehelin T and Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA **76**, 4350 – 4354 (1979)

24 Gelboin HV: Cytochrome P450 and monoclonal antibodies. Pharmacol Rev **45**, 413 – 453 (1993)

25 van Birgelen AP and van den Berg M: Toxicokinetics. Food Add Contam **17**, 267 – 273 (2000)

26 Schenkman JB and Jansson I: Interactions between cytochrome P450 and cytochrome b_5. Drug Metab Rev **31**, 351 – 364 (1999)