JPP
Journal of
Pharmacy and Pharmacology

JPP 2005, 57: 227–232
© 2005 The Authors
Received July 23, 2004
Accepted October 15, 2004
DOI 10.1211/0022357055489
ISSN 0022-3573

Alteration of the pharmacokinetics of theophylline by rutaecarpine, an alkaloid of the medicinal herb *Evodia rutaecarpa*, in rats

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Acknowledgement and funding: The authors thank Ms Shu-Yun Wang for help with the treatment of rats and determination of enzyme activity. This work was supported by the National Research Institute of Chinese Medicine and the grant NSC92-3112-B-077-001.

Abstract

Rutaecarpine is a main active alkaloid present in the medicinal herb, *Evodia rutaecarpa*. The cytochrome P450 (CYP) 1A2 substrate, theophylline, is an important therapeutic agent for the treatment of asthma, but has a narrow therapeutic index. To evaluate the pharmacokinetic interaction of theophylline with rutaecarpine, the effects of rutaecarpine on CYP1A2 activity and theophylline pharmacokinetics were investigated. Oral treatment of Sprague-Dawley rats with 50 mg kg^{-1} rutaecarpine for three days through a gastrogavage caused a 4- and 3-fold increase in liver microsomal 7-ethoxyresorufin *O*-deethylation (EROD) and 7-methoxyresorufin *O*-demethylation activity, respectively. In the kidney, rutaecarpine treatment caused a 3-fold increase in EROD activity. In the lungs, EROD activity was elevated from an undetectable to a detectable level by rutaecarpine. Pharmacokinetic parameters of theophylline were determined using a microdialysis sampling method. Rutaecarpine pre-treatment increased the clearance of theophylline in a dose-dependent manner. Pre-treatment of rats with 50 mg kg^{-1} rutaecarpine caused a 3-fold increase in theophylline clearance and a 70%, 68% and 68% decrease in the area under the concentration–time curve (AUC), mean residence time (MRT) and half-life, respectively. These results demonstrated that rutaecarpine treatment elevated CYP1A2 catalytic activity and theophylline excretion in rats. In patients taking theophylline, adverse effects might be noticed when a rutaecarpine-containing herbal preparation is used concomitantly.

Introduction

Microsomal cytochrome P450 (CYP)-dependent monooxygenase is the primary enzyme system responsible for the oxidation of drugs. This monooxygenase system consists of phospholipids, a family of CYP and NADPH-CYP reductase. Although herbal medicines have been widely used for centuries, it has been pointed out that the herb-mediated induction or inhibition of CYPs could diminish the pharmacological efficacy, or elevate the toxicity, of CYP drug substrates. Rutaecarpine is a main active alkaloid present in the medicinal herb, *Evodia rutaecarpa*. The unripe fruit of *E. rutaecarpa* (*E. fructus*) has been used in herbal preparations for the treatment of gastrointestinal disorders, headache and amenorrhoea (Tang & Eisenbrand 1992). One gram of aqueous and methanol extracts of *E. fructus* contains 0.03 and 7.6 mg rutaecarpine, respectively (Ueng et al 2002a). Rutaecarpine has been reported to compete with 2,3,7,8-tetrachlorodibenzo-*p*-dioxine (TCDD) for binding to the Ah receptor, which plays an important role in the transcriptional induction of CYP1A (Gillner et al 1989; Nebert et al 1993). Our previous report showed that rutaecarpine was a CYP1A inducer in mouse liver and kidney (Ueng et al 2001). 7-Ethoxyresorufin and 7-methoxyresorufin are commonly used as model substrates of CYP1A; rutaecarpine treatment increased mouse hepatic 7-ethoxyresorufin *O*-deethylation (EROD) and 7-methoxyresorufin *O*-demethylation and the renal EROD activity. However, the effects of rutaecarpine on rat hepatic and extrahepatic CYP1A were not reported.

The alkaloid theophylline is a potent bronchodilator that has been used for the treatment of acute asthma (Weinberger & Hendeles 1996; Barnes 2003). However,

theophylline has a narrow therapeutic index in man. The bioavailability of theophylline through oral administration is close to unity in man and rats (Teunissen et al 1985). In man, theophylline is metabolized through *N*-demethylation and 8-oxidation, with the 8-oxidation being dominant (Robson et al 1988; Ha et al 1995; Tjia et al 1996). In rats, theophylline is 8-oxidized by CYP1A2 and CYP2B1 to form 1,3-dimethyluric acid (1,3-DMU) and CYP1A2 has higher catalytic activity (Williams et al 1979; Salyers et al 1994). Drugs such as macrolide antibiotics, ciprofloxacin, allopurinol and cimetidine, that interfere with hepatic metabolism, decrease the clearance of theophylline (Barnes 2003). Monitoring of plasma levels may be necessary, especially in elderly patients treated with multiple drugs. Asthma patients need long-term health care. In Asia, compound herbal medicines are commonly used as alternative medicines, especially for the treatment of chronic disease. *E. fructus* is frequently used for the treatment of headache and gastrointestinal-disorder-induced abdominal pain. *E. fructus* may be added in compound medicine, which is used in theophylline-treated asthma patients with these symptoms. It is also possible that theophylline is used in patients pre-exposed to rutaecarpine for a period of time. To demonstrate the pharmacokinetic interaction of theophylline with rutaecarpine, we have studied the effect of rutaecarpine on CYP1A2 catalytic activity and determined the pharmacokinetic parameters of theophylline using microdialysis in rats.

Materials and Methods

Chemicals and reagents

Rutaecarpine was synthesized from tryptamine as described previously (Don et al 2003). The purity of rutaecarpine was higher than 99% as analysed by HPLC. 7-Ethoxyresorufin, 7-methoxyresorufin and theophylline were purchased from Sigma Chemicals (St Louis, MO). Acetonitrile and methanol were purchased from Merck Co. (Darmstadt, Germany).

Animal treatment

Male Sprague-Dawley rats, aged 4–6 weeks, 150–200 g, were obtained from the Laboratory Animal Center of the National Yang-Ming University, Taipei. The institutional animal experimentation committee of the National Research Institute of Chinese Medicine reviewed and approved all experimental protocols involving animals. Rats had free access to laboratory rodent diet (no. 5P14; PMI Feeds Inc., Richmond, IN) and water. Before experiment, rats were allowed a one-week acclimation in the animal centre with air conditioning (25 ± 1°C) and an automatically controlled photoperiod of 12 h light daily. Rutaecarpine was dissolved in corn oil. Rats were treated with rutaecarpine 25 or 50 mg kg^{-1} daily by gastrogavage for 3 days. In the control group, rats received the same amount of corn oil without rutaecarpine.

Microsomal preparation and activity determination

Rat tissues were removed 24 h after the last treatment with rutaecarpine. Tissue microsomes were prepared from individual rats by differential centrifugation at 4°C (Alvares & Mannering 1970). The CYP content was determined using the CO-difference spectral method of Omura & Sato (1964). The *O*-dealkylation of 7-ethoxyresorufin and 7-methoxyresorufin was determined by measuring the fluorescence of resorufin (Pohl & Fouts 1980). 7-Ethoxycoumarin *O*-deethylation activity was determined by measuring the fluorescence of hydroxycoumarin (Greenlee & Poland 1978). Microsomal protein concentration was determined using bovine serum albumin as a standard (Lowry et al 1951).

Microdialysis

Rats were initially anaesthetized with 1 g kg^{-1} urethane and 0.1 g kg^{-1} α-chloralose intraperitoneally and remained anaesthetized throughout the experimental period. Rat body temperature was maintained at 37°C using a heating pad during the experiment. Blood microdialysis system comprised a CMA/100 microinjection pump (CMA, Stockholm, Sweden) and microdialysis probes. The dialysis probe was prepared as described previously (Tsai et al 1999) and was inserted into the jugular vein/right atrium. The anticoagulant dextrose (ACD) solution was perfused through the probe at a flow rate of 2 μL min^{-1}. The ACD solution contained 3.5 mM citric acid, 7.5 mM sodium citrate and 13.6 mM dextrose. Outflow blood dialysates were collected every 10 min using a fraction collector (CMA/140). Following a 2-h stabilization period after probe implantation, theophylline was administered via the femoral vein and its concentration in the dialysate was determined by HPLC on the same experimental day. For determination of the in-vivo recovery, a retrograde calibration technique was used. An ACD solution containing theophylline was perfused through the probe at a constant flow rate of 2 μL min^{-1}. The theophylline concentration in the perfusate (C_{perf}) and dialysate (C_{dial}) was determined by HPLC. The in-vivo recovery ratio (R_{dial}) of theophylline across the microdialysis probe was calculated by the following equation: $R_{dial} = (C_{perf} - C_{dial})/C_{perf}$. The theophylline concentration (C_m) in the dialysate was converted to unbound concentration (C_u) as follows: $C_u = C_m/R_{dial}$.

Pharmacokinetic analysis

Six hours after the last treatment with rutaecarpine, access to the diet was removed and only water was provided. Eighteen hours later, the rats were treated with theophylline 3 mg kg^{-1} via the femoral vein for pharmacokinetic experiments. The theophylline concentration was determined by HPLC using a Phenomenex LUNA microbore Phenyl-Hexyl column (150×1 mm; particle size 5 μm; Torrance, CA). The mobile phase consisted of acetonitrile–methanol–10 mM sodium phosphate buffer, pH 4.0 (10:20:70, v/v). Separation was carried out at a flow rate of 0.05 mL min^{-1} and theophylline was detected by measuring the absorbance at 270 nm. Pharmacokinetic calculation of

the data set from individual rats was performed using the software WinNonlin Standard Edition Version 1.1 (Scientific Consulting Inc., Apex, NC) by a non-compartmental method. The area under the concentration–time curve (AUC), the area under the first moment curve (AUMC) and the mean residence time (MRT) were calculated by statistical moments (Gabrielsson & Weiner 1994). The AUCs from time zero to infinity ($AUC_{0\text{-inf}}$) were calculated by the trapezoidal rule and extrapolated to time infinity by the addition of $AUC_{t\text{-inf}}$. An analogous method was employed to calculate the AUMC as follows:

$$AUC = AUC_{0\text{-t}} + AUC_{t\text{-inf}} = AUC_{0\text{-t}} + C_{last}/\lambda_z \quad (1)$$

$$AUMC = AUMC_{last} + (t_{last} \cdot C_{last}/\lambda_z) + C_{last}/(\lambda_z)^2 \quad (2)$$

where C_{last} and t_{last} are the last observed concentration and time, respectively, and λ_z is the terminal slope estimated by linear regression of the logarithmic value of the last observed data. The clearance (CL) and MRT were calculated as follows: CL = dose/AUC and MRT = AUMC/AUC.

Statistical analysis

The statistical analysis was performed with SPSS version 10.0 (SPSS Inc., Chicago, IL). The differences between >2 sets of data (the control and groups treated with various doses of rutaecarpine) were analysed by one-way analysis of variance followed by Dunnett's test for multiple comparisons. The statistical significance of differences between control and treated groups was evaluated by Student's *t*-test. $P < 0.05$ was considered as statistically significant.

Results and Discussion

To determine the dose–response relationship of the effect of rutaecarpine on rat hepatic EROD and 7-methoxyresorufin *O*-demethylation activity, rats were treated with various doses of rutaecarpine for three days. Rutaecarpine treatment had no effect on CYP content at doses up to 75 mg kg^{-1} (Figure 1). However, rutaecarpine treatment caused dose-dependent increases in rat liver microsomal EROD and 7-methoxyresorufin *O*-demethylation activity. Treatment with 10, 25, 50 and 75 mg kg^{-1} rutaecarpine caused a 3-, 6-, 4- and 6-fold increase in EROD activity, respectively. Treatment with 25, 50 and 75 mg kg^{-1} rutaecarpine caused a 2-, 3- and 3-fold increase in 7-methoxyresorufin *O*-demethylation activity, respectively. Rutaecarpine treatment at 25–75 mg kg^{-1} caused an induction of these activities at the plateau level. Thus, rats were treated with 50 mg kg^{-1} rutaecarpine daily for three days in the subsequent studies of hepatic and extrahepatic CYP activity. Treatment with rutaecarpine had no effect on CYP and cytochrome b_5 contents or on NADPH-CYP reductase activity in rat liver, kidney and lung microsomes (Table 1). Rutaecarpine treatment caused a 4- and 3-fold increase in liver microsomal EROD and 7-methoxyresorufin *O*-demethylation activity, respectively. In kidney, rutaecarpine treatment increased EROD activity 3 fold. In the lungs, EROD activity was increased from an undetectable to a detectable level. However, the hepatic, renal and pulmonary 7-ethoxycoumarin *O*-deethylation activity was not affected by rutaecarpine treatment. Our results demonstrated that rutaecarpine treatment increased rat CYP1A activity not only in the liver but also in the kidneys and lungs. However, the induction might not be strong enough to show significant difference in tissue weight and total CYP content determined spectrally. The effect of rutaecarpine on other rat CYP forms was not clear. Rutaecarpine is known to be a competitor of TCDD for the binding to Ah receptors (Gillner et al 1989). In mouse study, our results showed that hepatic CYP1A2 protein and mRNA levels were stimulated by rutaecarpine (Ueng et al., 2001; unpublished results). The CYP1A induction may be triggered by the Ah receptor-mediated signalling pathway. However, in both mouse and human liver microsomes, our report revealed that rutaecarpine

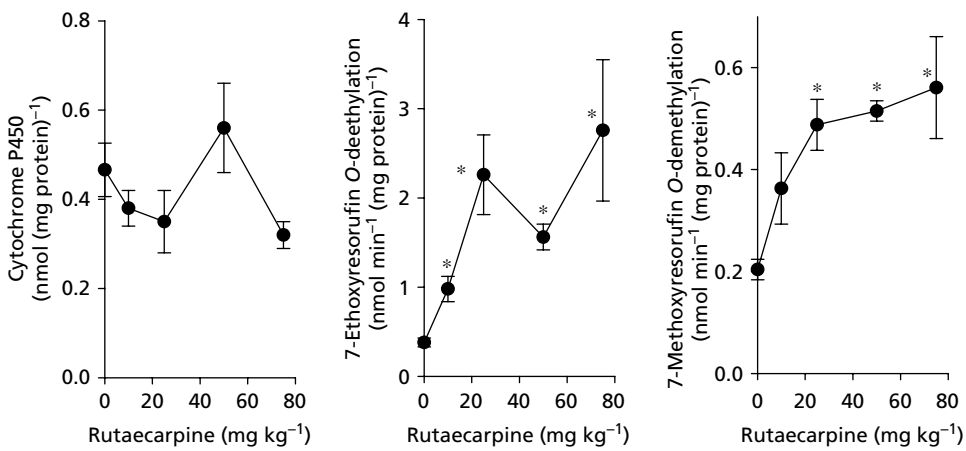

Figure 1 Dose–response of the effects of rutaecarpine on rat liver microsomal cytochrome P450 content and 7-methoxyresorufin *O*-demethylation and 7-ethoxyresorufin *O*-deethylation activity. Results represent the mean ± s.e.m. of three rats. *$P < 0.05$ vs control values.

Table 1 Effects of rutaecarpine on microsomal cytochrome P450 content and activity in rat liver, kidney and lungs

	Cytochrome P450 (nmol mg^{-1})	Cytochrome b_5 (nmol mg^{-1})	NADPH-cytochrome P450 reductase (nmol min^{-1} mg^{-1})	7-Ethoxyresorufin *O*-deethylation (pmol min^{-1} mg^{-1})	7-Methoxyresorufin *O*-demethylation (pmol min^{-1} mg^{-1})	7-Ethoxycoumarin *O*-deethylation (nmol min^{-1} mg^{-1})
Liver						
Control	0.47 ± 0.06	0.44 ± 0.03	159 ± 10	383 ± 53	205 ± 21	1.72 ± 0.13
Rutaecarpine	0.56 ± 0.10	0.44 ± 0.06	142 ± 20	1562 ± 144*	515 ± 22*	2.41 ± 0.43
Kidney						
Control	0.03 ± 0.01	0.04 ± 0.01	35.3 ± 3.2	19.9 ± 7.1	2.47 ± 0.64	0.18 ± 0.01
Rutaecarpine	0.02 ± 0.00	0.04 ± 0.00	36.9 ± 3.0	61.2 ± 6.0*	2.61 ± 0.29	0.28 ± 0.03
Lung						
Control	0.02 ± 0.00	0.05 ± 0.00	27.0 ± 5.0	n.d.	n.d.	0.01 ± 0.00
Rutaecarpine	0.02 ± 0.01	0.05 ± 0.00	22.4 ± 3.0	5.9 ± 1.1	n.d.	0.01 ± 0.00

Rats were treated with 50 mg kg^{-1} rutaecarpine daily through gastrogavage for three days. Tissue microsomes were prepared and monooxygenase activity was determined as described in Materials and Methods. Results represent the mean ± s.e. of three rats in a group. *$P < 0.05$ vs control. n.d., not detectable.

was a selective CYP1A2 inhibitor (Ueng et al 2002b). Our result of structural modelling showed a good fitting of rutaecarpine with the putative active site of CYP1A2 (Don et al 2003). These results suggested that rutaecarpine interfered in the binding of alkoxyresorufin to CYP1A2. The absence of Ah receptor-mediated signalling and other cellular regulatory pathways in the microsomes can contribute to the difference in the in-vitro and in-vivo effects.

CYP1A catalyses the oxidation of many therapeutic agents, such as paracetamol (acetaminophen), tamoxifen, phenazone (antipyrin) and theophylline. The CYP1A subfamily is also responsible for the activation of many environmental carcinogens, such as benzo(a)pyrene and 2-aminoanthracene. CYP1A members are expressed in several tissues including liver, kidney and lungs. In general, hepatic CYP1A plays a main role in the oxidation of therapeutic substrates. However, pulmonary CYP1A is closely associated with the bioactivation of airborne toxicants from cigarette smoke, motorcycle exhaust, frying-meat emission particulate and other sources (Ueng et al 1998; Alexandrov et al 2002; Wang et al 2003). The CYP1A induction by rutaecarpine in these tissues suggested that rutaecarpine treatment might elevate the risk of the toxicity of pro-toxicants in tissues.

Theophylline, a CYP1A2 substrate, is an important therapeutic agent with a narrow therapeutic index. Human hepatic CYP1A2 catalyses the *N*-demethylation and 8-oxidation of theophylline. The dominant metabolic pathway of theophylline is 8-oxidation and CYP1A2 had a low K_m value for this reaction (Zhang & Kaminsky 1995). Different pharmacokinetic parameters could be obtained when different sample collection and anaesthetic methods were used (Telting-Diaz et al 1992). A non-linear pharmacokinetics of theophylline was observed at the dose higher than 10 mg kg^{-1} in rats (Teunissen et al 1985). Our results showed that microdialysis sampling method provides a useful tool to determine the kinetic parameters of theophylline at the low dose of 3 mg kg^{-1}.

In the pharmacokinetic analysis, the in-vivo recovery of theophylline in blood (1 μg mL^{-1}) was 0.77 ± 0.01 (mean ± s.e., n = 6). The concentration–time curve of theophylline in blood is shown in Figure 2. Rutaecarpine pre-treatment significantly changed the pharmacokinetic profiles of theophylline at the dosages of 25 and 50 mg kg^{-1} rutaecarpine daily for three days (Table 2). Pre-treatment with 25 mg kg^{-1} rutaecarpine caused a 26%, 54% and 53% decrease in AUC, MRT and half-life of theophylline,

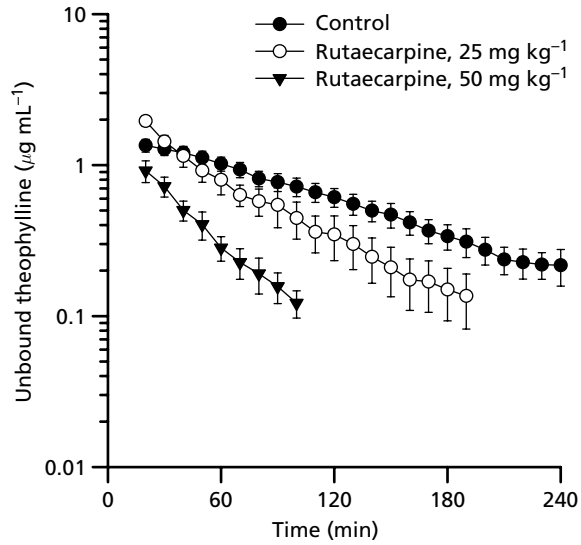

Figure 2 Mean unbound theophylline concentration–time curves in rat blood after intravenous treatment with 3 mg kg^{-1} theophylline. Rats were pre-treated with 25 (○) or 50 (▼) mg kg^{-1} rutaecarpine daily for three days and then theophylline was injected via the femoral vein on the fourth day. Control group (●) received corn oil only instead of rutaecarpine before theophylline treatment. Results are presented as the mean ± s.e.m. of five rats.

Table 2 Pharmacokinetic parameters of theophylline in the control and the rutaecarpine-pre-treated groups of rats

Parameters	Control	Rutaecarpine 25 mg kg^{-1} daily	Rutaecarpine 50 mg kg^{-1} daily
AUC (min μg mL^{-1})	196.1 ± 26.4	144.7 ± 26.0*	58.4 ± 9.8*,#
CL (mL kg^{-1} min^{-1})	17.3 ± 3.1	22.7 ± 2.8*	58.5 ± 10.9*,#
MRT (min)	114 ± 11	53 ± 9*	36 ± 3*
t½ (min)	79 ± 7	37 ± 6*	25 ± 2*

Rats were pre-treated with rutaecarpine at 25 or 50 mg kg^{-1} daily through gastrogavage for three days. On the fourth day, rats were treated with theophylline at 3 mg kg^{-1} via femoral vein injection. Results represent mean ± s.e. of five rats in control and rutaecarpine-treated groups. *$P < 0.05$ vs control; #$P < 0.05$ vs group pre-treated with rutaecarpine 25 mg kg^{-1} daily.

respectively. Pre-treatment with 50 mg kg^{-1} rutaecarpine caused a 70%, 68% and 68% decrease in AUC, MRT and half-life of theophylline, respectively. Consistent with these changes, the clearance of theophylline was increased by rutaecarpine. The changes in pharmacokinetic parameters showed a dose dependence. At the initial time point of dialysate collection (20 min), the blood theophylline concentration was higher in 25 mg kg^{-1} rutaecarpine-treated rats than that in the control group. The reason for this transient increase in blood concentration was not clear. However, this increase occurred only at this single time point without affecting the dose-dependent decrease in the AUC of theophylline by rutaecarpine. Our results revealed that pre-treatment of rats with rutaecarpine stimulated the excretion of theophylline. The AUC values of theophylline in groups treated with 25 and 50 mg kg^{-1} rutaecarpine were significantly lower than the values in the control group. To date, no serious drug interaction by *E. fructus* has been reported. There are several formulations of theophylline, including sustained-release and immediate-release formulations (Hendeles et al 1984). The pharmacokinetic parameters of oral theophylline are greatly influenced by the formulation and brand studied. The drug interaction caused by rutaecarpine may be different due to the differences in the formulations of theophylline. In man, the daily dose of *E. fructus* is about 0.1–0.3% of the doses used to treat rats in this study (Zhu 1998). Thus, the effect of rutaecarpine on human metabolism of CYP1A2 substrate needs further studies in-vivo.

Conclusion

In summary, our results demonstrated that rutaecarpine treatment increased CYP1A activity in rat liver, kidney and lungs. In consensus with the CYP1A induction, pre-treatment with rutaecarpine increased the clearance and decreased the blood concentration of theophylline. Factors such as species difference and formulational changes of theophylline may vary this interaction. Possible adverse effects should be looked for when patients take theophylline concomitantly with rutaecarpine-containing herbal preparations.

References

Alexandrov, K., Cascorbi, I., Rojas, M., Bouvier, G., Kriek, E., Bartsch, H. (2002) CYP1A1 and GSTM1 genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. *Carcinogenesis* **23**: 1969–1977

Alvares, A. P., Mannering, G. J. (1970) Two-substrate kinetics of drug-metabolizing enzyme systems of hepatic microsomes. *Mol. Pharmacol.* **6**: 206–211

Barnes, P. J. (2003) Therapy of chronic obstructive pulmonary disease. *Pharmacol. Ther.* **97**: 87–94

Don, M.-J., Lewis, D. F. V., Wang, S.-Y., Tsai, M.-W., Ueng, Y.-F. (2003) Effects of structural modification on the inhibitory selectivity of rutaecarpine derivatives on human CYP1A1, CYP1A2, and CYP1B1. *Bioorg. Med. Chem. Lett.* **13**: 2535–2538

Gabrielsson, J., Weiner, D. (1994) Non-compartmental analysis. In: *Pharmacokinetic and pharmacodynamic data analysis concepts and applications*. Swedish Pharm. Press, Stockholm, pp 621–626

Gillner, M., Bergman, J., Cambillau, C., Gustafsson, J. A. (1989) Interactions of rutaecarpine alkaloids with specific binding sites for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver. *Carcinogenesis* **10**: 651–654

Greenlee, W. F., Poland, A. (1978) 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

Ha, H. R., Chen, J., Freiburghaus, A. U., Follath, F. (1995) Metabolism of theophylline by cDNA-expressed human cytochrome P-450. *Br. J. Clin. Pharmacol.* **39**: 321–326

Hendeles, L., Iafrate, R. P., Weinberger, M. (1984) A clinical and pharmacokinetic basis for the selection and use of slow release theophylline products. *Clin. Pharmacokinet.* **9**: 95–135

Lowry, O. H., Roseborough, N. J., Farr, A. L., Randall, R. L. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275

Nebert, D. W., Puga, A., Vasiliou, V. (1993) Role of the Ah receptor and the dioxine-inducible [Ah] gene battery in toxicity, cancer, and signal transduction. *Ann. N. Y. Acad. Sci.* **685**: 624–640

Omura, T., Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemeprotein nature. *J. Biol. Chem.* **239**: 2370–2379

Pohl, R. J., Fouts, J. R. (1980) A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal. Biochem.* **107**: 150–155

Robson, R. A., Miners, J. O., Matthews, A. P., Stupans, I., Meller, D., McManus, M. E., Birkett, D. J. (1988) Characterization of theophylline metabolism by human liver microsomes. Inhibition and immunochemical studies. *Biochem. Pharmacol.* **37**: 1651–1659

Salyers, K. L., Barr, J., Sipes, I. G. (1994) In vitro metabolism of theophylline by rat and human liver tissue. *Xenobiotica* **24**: 389–399

Tang, W., Eisenbrand, G. (1992) Evodia rutaecarpa (Juss) Benth. In: Tang, W., Eisenbrand, G. (eds) *Chinese drugs of plant origin*. Springer-Verlag, Berlin, pp 509–514

Telting-Diaz, M., Scott, D. O., Lunte, C. E. (1992) Intravenous microdialysis sampling in awake, freely-moving rats. *Anal. Chem.* **64**: 806–810

Teunissen, M. W., Brorens, I. O., Geerlings, J. M., Breimer, D. D. (1985) Dose-dependent elimination of theophylline in rats. *Xenobiotica* **15**: 165–171

Tjia, J. F., Colbert, J., Back, D. J. (1996) Theophylline metabolism in human liver microsomes: inhibition studies. *J. Pharmacol. Exp. Ther.* **276**: 912–917

Tsai, T. H., Chen, Y. F., Chen, I. F., Chen, C. F. (1999) Measurement of unbound caffeic acid in rat blood by on-line microdialysis coupled with liquid chromatography and its application to pharmacokinetic studies. *J. Chromatogr. B* **729**: 119–125

Ueng, T.-H., Hwang, W.-P., Chen, R.-M., Wang, H.-W., Kuo, M.-L., Park, S. S., Guengerich, F. P. (1998) Effects of motorcycle exhaust on cytochrome P-450-dependent monooxygenases and glutathione S-transferase in rat tissues. *J. Toxicol. Environ. Health A* **54**: 509–527

Ueng, Y.-F., Wang, J.-J., Lin, L.-C., Park, S. S., Chen, C.-F. (2001) 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

Ueng, Y.-F., Ko, H.-C., Chen, C.-F., Wang, J.-J., Chen, K.-T. (2002a) Modulation of drug-metabolizing enzymes by extracts of a herbal medicine *Evodia rutaecarpa* in C57BL/6J mice. *Life Sci.* **71**: 1267–1277

Ueng, Y.-F., Jan, W.-C., Lin, L.-C., Chen, T.-L., Guengerich, F. P., Chen, C.-F. (2002b) The alkaloid rutaecarpine is a selective inhibitor of cytochrome P450 1A in mouse and human liver microsomes. *Drug Metab. Dispos.* **30**: 349–353

Wang, H.-W., Ueng, T.-H., Chen, T.-L., Yang, P.-C. (2003) Effect of frying-meat emission particulate on 17beta-estradiol 2- and 4-hydroxylation in human lung adenocarcinoma CL5 cells. *J. Toxicol. Environ. Health A* **66**: 1175–1188

Weinberger, M., Hendeles, L. (1996) Theophylline in asthma. *N. Engl. J. Med.* **334**: 1380–1388

Williams, J. F., Lowitt, S., Szentivanyi, A. (1979) Effects of phenobarbital and 3-methylcholanthrene pretreatment on the plasma half-life and urinary excretion profile of theophylline and its metabolites in rats. *Biochem. Pharmacol.* **28**: 2935–2940

Zhang, Z.-Y., Kaminsky, L. S. (1995) Characterization of human cytochrome P450 involved in theophylline 8-hydroxylation. *Biochem. Pharmacol.* **50**: 205–211

Zhu, Y.-P. (1998) *Chinese materia medica: chemistry, pharmacology and applications*. Harwood Academic publishers, Amsterdam, The Netherlands, pp 356–360