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Effects of Rutaecarpine on the Metabolism and Urinary Excretion of Caffeine in Rats

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Although rutaecarpine, an alkaloid originally isolated from the unripe fruit of *Evodia rutaecarpa*, has been reported to reduce the systemic exposure of caffeine, the mechanism of this phenomenon is unclear. We investigated the microsomal enzyme activity using hepatic S-9 fraction and the plasma concentration-time profiles and urinary excretion of caffeine and its major metabolites after an oral administration of caffeine in the presence and absence of rutaecarpine in rats. Following oral administration of 80 mg/kg rutaecarpine for three consecutive days, caffeine (20 mg/kg) was given orally. Plasma and urine were collected serially for up to 24 h and the plasma and urine concentrations of caffeine and its metabolites were measured, and compared with those in control rats. The areas under the curve of both caffeine and its three major metabolites (paraxanthine, theophylline, and theobromine) were significantly reduced by rutaecarpine, indicating that caffeine was rapidly converted into the desmethylated metabolites, and that those were also quickly transformed into further metabolites via the hydroxyl metabolites due to the remarkable induction of CYP1A2 and 2E1. The significant induction of ethoxyresorufin *O*-deethylase, pentoxyresorufin *O*-dependentylase, and *p*-nitrophenol hydroxylase strongly supported the decrease in caffeine and its major metabolites in plasma, as well as in urine. These results clearly suggest that rutaecarpine increases the metabolism of caffeine, theophylline, theobromine, and paraxanthine by inducing CYP1A2 and CYP2E1 in rats.

Key words: Caffeine, Metabolites, Rutaecarpine, Cytochrome P450, Rat

INTRODUCTION

Caffeine is widely used in soft drinks and numerous prescription and over-the-counter drugs. It is absorbed from the digestive tract and rapidly distributed throughout all tissues. It induces hepatic cytochrome P450 (CYP), including CYP1A2 in rodents (Chen et al., 1996). Interestingly, caffeine is oxidized at distinct structural positions by different CYPs: CYP1A2 catalyzes 3-N-demethylation to paraxanthine and CYP2E1 catalyzes 1-N-demethylation to theobromine and 7-N-demethylation to theophylline (Caubet et al., 2004).

In Korea, herbal remedies are very popular as alternative medicines. The fruit of *Evodia rutaecarpa*, has long been used in herbal preparations for treating gastrointestinal disorders, headache, and amenorrhea (Ueng et al., 2002; Lee et al., 2004a). Rutaecarpine (8,13-dihydro-7H-indolo-[2',3':3,4]-pyrido-[2,1-b]-quinazolin-5-one) is an alkaloid originally isolated from the unripe fruit of *Evodia rutaecarpa* (Chiou et al., 1996) that has anti-inflammatory activity via cyclooxygenase-2 inhibition (Moon et al., 1999; Woo et al., 2001).

Rutaecarpine induces CYP1A, 2B, and 2E1 in mice based on measuring CYP-associated enzyme activities (Ueng et al., 2001; Lee et al., 2004a). Rutaecarpine probably interacts with CYP substrates, although only a

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few works have investigated drug-drug interactions, e.g., rutaecarpine alters the pharmacokinetics of theophylline in rats (Ueng et al., 2005).

Although rutaecarpine has been reported to reduce the systemic exposure of caffeine (Tsai et al., 2005), the mechanism of this phenomenon is unclear. This work investigated the microsomal enzyme activity using hepatic S-9 fraction and the plasma concentration-time profiles and urinary excretion of caffeine and its three major metabolites (theophylline, theobromine, and paraxanthine) after an oral administration of caffeine in rutaecarpine-pretreated rats.

MATERIALS AND METHODS

Materials

Rutaecarpine (purity, >99%) used in this study was synthesized by our group (Lee et al., 2001). Caffeine, ethoxyresorufin, pentoxyresorufin, *p*-nitrophenol, erythromycin, and the reduced form of β-nicotinamide adenine dinucleotide phosphate (β-NADPH) were purchased from Sigma Chemical. Methanol and acetonitrile were HPLC-grade from Merck. All other chemicals were of analytical grade.

Animals

Specific pathogen-free male Sprague-Dawley rats (250-280 g) were obtained from The Orient Co. Animals were received at 6 weeks of age and acclimated for at least one week. Upon arrival, the animals were randomized and housed three per cage in strictly controlled conditions of 23 ± 3°C and 50 ± 10% relative humidity. A 12-h light/dark cycle was used with an intensity of 150-300 Lux. All animal procedures were approved by the Institutional Animal Care and Use Committee of Yeungnam University College of Pharmacy based on the guiding principles in 'The Use of Animals in Toxicology' recommended by the Society of Toxicology.

Animal treatment

The rats were divided randomly into two groups, without (control, n = 5) and with (n = 5) oral rutaecarpine. The rats were given oral rutaecarpine dissolved in corn oil at a dose of 80 mg/kg/day, once a day, for three consecutive days, followed by an oral administration of 20 mg/kg caffeine one day after the last dose of rutaecarpine. The control group received 10 mL/kg of corn oil, followed by the same dose of caffeine.

One day before the administration of caffeine, the rat was anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg). Jugular vein was cannulated with a polyethylene tube (PE-50 tube, inner diameter, 0.58 mm; outer diameter, 0.96 mm, Natume Seisakusho) filled with heparin (50 IU/mL). Blood samples (200 μL) were taken via the jugular vein immediately before and 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, and 24 h after the administration of caffeine. The blood samples were centrifuged at 3,000 × g for 20 min at 4°C to prepare plasma samples. Urine samples were serially collected for every 4 h up to 12 h, and for the following 12 h. After measuring the volumes of urine samples, those were stored at a deep freezer together with the plasma samples until analyzed.

Preparation of liver S-9 fraction

Rutaecarpine (80 mg/kg) and corn oil were given orally to measure the liver S-9 protein in another two groups of rats. All rats were necropsied 24 h after the last dose. The liver was perfused with ice-cold saline via the hepatic portal vein. Then, the liver was removed and homogenized with four volumes of ice-cold 0.1 M potassium phosphate buffer, pH 7.4. The liver S-9 fraction was isolated by centrifugation at 9,000 × g for 20 min at 4°C and stored at −80°C until use (Lee et al., 2004a). The liver S-9 protein content was determined using bovine serum albumin as a standard (Lowry et al., 1951).

Cytochrome P450 activity

Ethoxyresorufin *O*-deethylase (EROD) activity was determined, as previously described, with a slight modification (Blank et al., 1987). The reaction mixture consisted of 0.1 M potassium phosphate buffer, pH 7.4, containing 2 mg/mL of bovine serum albumin, 5 mM glucose-6-phosphate, 1 U of glucose-6-phosphate dehydrogenase, 5 μM NADPH, and 2.5 μM 7-ethoxyresorufin. The formation of resorufin was monitored fluorometrically at an excitation maximum of 550 nm and an emission maximum of 585 nm. Pentoxyresorufin *O*-dependentylase (PROD) activity was determined according to a described method (Lubet et al., 1985) with 2.0 μM pentoxyresorufin. *p*-Nitrophenol hydroxylase (PNPH) activity was determined as described elsewhere (Koop et al., 1986). The reaction mixture consisted of 0.1 M potassium phosphate buffer, pH 7.4, containing 100 μM *p*-nitrophenol, 1 mM NADPH, and an enzyme source. The amount of 4-nitrocatechol formed was measured spectrophotometrically at 512 nm. Erythromycin N-demethylase (ERDM) activity was determined by measuring the amount of formaldehyde formed, as described previously (Nash, 1953). Erythromycin at 400 μM was used as a substrate for assaying ERDM.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Sample preparation

First, 90 μL of internal standard solution (IS, 1 μg thiamphenicol in 1 mL methanol) was added to 30 μL of plasma. After vortexing for 10 min and centrifuging at 15,000 × g at 15°C to remove proteins, 3 μL of supernatant was used for the LC-MS analysis.

Instrument

High-performance liquid chromatography (HPLC) was performed on the Agilent 1100 system (Agilent Technologies) with an API4000 trap mass spectrometer (SCIEX Division of MDS) equipped with an electrospray ionization (ESI) source. The Zorbax SB C_{18} (2.1 × 150 mm, 2.5 μm) column was used for separation. The column temperature was maintained at 35°C. The mobile phase consisted of acetonitrile and 0.05% acetic acid in distilled water (15:85%, v/v). An isocratic program was used for the HPLC separation with a flow rate of 0.25 mL/min. The volume of injection was 3 μL. The mass transitions used for caffeine, paraxanthine, and theobromine were positive ion mode with *m/z* 194.8 → 137.8, 180.8 → 124.0, and 180.8 → 137.8, declustering potential at 58, 51, and 61 V, entrance potential all at 10 V, collision energy of 28, 27, and 25 V, and collision exit potential at 8, 10, and 12 V, respectively. Theophylline and thiamphenicol (IS) were detected using the negative ion mode of *m/z* 178.8 → 163.6 and 354.1 → 289.7, declustering potential at −55 and −74 V, entrance potential all at −10 V, collision energy of −28 and −17 V, and collision exit potential at −9 and −7 V, respectively. Nitrogen was used as the collision gas for the tandem mass spectrometry experiments, followed by the isolation of ions over a selected mass window of 1 Da. The lower limits of quantitation of caffeine and its primary metabolites were 10 ng/mL in urine and plasma, except 50 ng/mL for paraxanthine in urine.

Pharmacokinetic analysis

Area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule-extrapolation method (Chiou et al., 1996). Standard methods were used to calculate the following pharmacokinetic parameters using non-compartmental analysis (WinNonlin; version 2.1; Scientific Consulting): maximum plasma concentration (C_{max}), AUC, elimination rate constants (k), and terminal half-life ($t_{1/2}$). The amount (μg) of caffeine and three metabolites excreted in urine was calculated as urine volume (mL) × concentration (μg/mL). The cumulative amounts excreted were plotted at the mean time of the collection. The results obtained were expressed as mean ± S.D. The

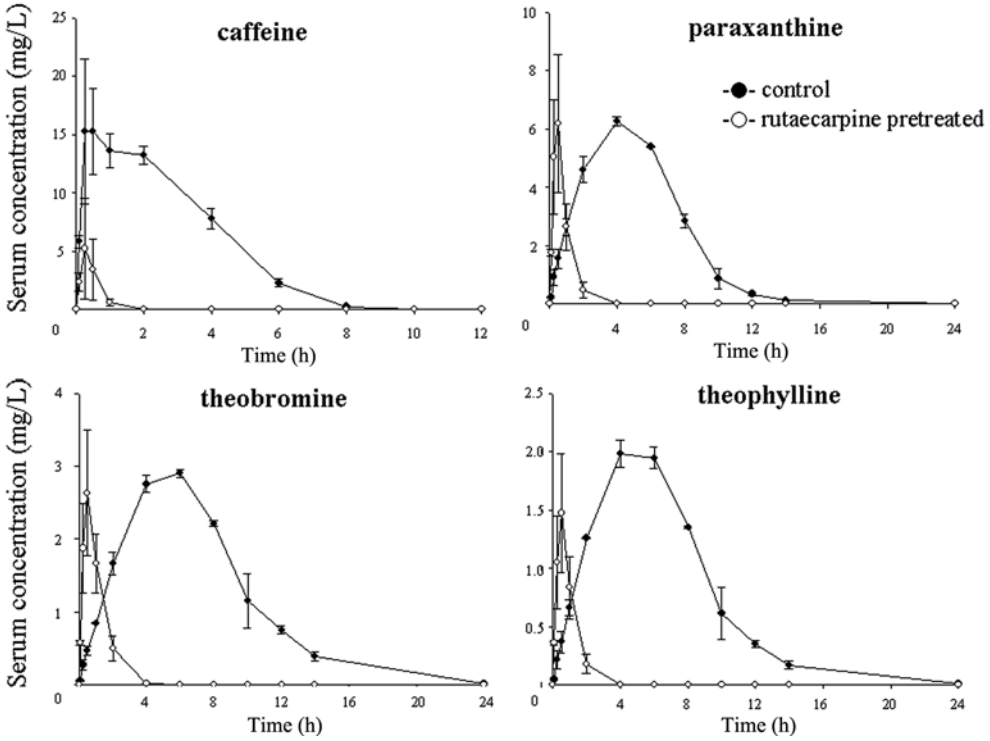

Fig. 1. Time course of the plasma concentrations of caffeine, paraxanthine, theobromine, and theophylline following an oral administration of caffeine in the presence and absence of rutaecarpine in rats. Each value represents the mean ± S.D. (n = 5).

statistical significance of the results was analyzed using Student's *t*-test, with *p* values of less than 0.05 considered statistically significant.

RESULTS

Fig. 1 shows the mean plasma concentration-time curve of caffeine and its three metabolites, theobromine, theophylline, and paraxanthine, in the presence and absence of rutaecarpine. The pharmacokinetic parameters of each compound are summarized in Table I. The mean plasma concentration of caffeine reached a C_{max} of 16.7 ± 4.19 μg/mL at 1.12 ± 1.20 h, and decayed with a half-life of 0.73 ± 0.07 h, and the AUC_{24h} was 60.2 ± 5.03 μg·h/mL. Three N-demethylated metabolites appeared continuously in plasma, and reached peaks at 4~6 h; the C_{max} and AUC_{24h} were 6.27 ± 0.16 μg/mL and 41.8 ± 1.28 μg·h/mL for paraxanthine, 2.90 ± 0.05 μg/mL and 25.4 ± 1.16 μg·h/mL for theobromine, and 1.98 ± 0.11 μg/mL and 16.2 ± 0.18 μg·h/mL for theophylline, respectively. The mean elimination half-life of paraxanthine was about 3 h, which was slightly longer than those of theobromine (2.30 h) and theophylline (2.54 h).

Rutaecarpine significantly reduced the systemic exposure of caffeine: the C_{max}, half-life, and AUC_{24h} of caffeine decreased by 68.8, 63.0, and 94.8% of those in the control, respectively. In the presence of rutaecarpine, the three metabolites appeared in plasma much faster than in the control, and the T_{max} was remarka-

Table I. Pharmacokinetic parameters of caffeine and its three metabolites, paraxanthine, theobromine, and theophylline, following the oral administration of 20 mg/kg caffeine in the presence and absence of rutaecarpine in rats

Parameter	Caffeine		Paraxanthine		Theobromine		Theophylline	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
C_{max} (μg/mL)	16.7 ± 4.19	5.22 ± 4.35*	6.27 ± 0.16	6.18 ± 2.37	2.90 ± 0.05	2.63 ± 0.86	1.98 ± 0.11	1.47 ± 0.51
T_{max} (hr	1.12 ± 1.20	0.25 ± 0.02	4.01 ± 0.04	0.54 ± 0.03**	6.02 ± 0.03	0.52 ± 0.04**	4.01 ± 0.04	0.54 ± 0.03**
AUC_{24h} (μg·h/mL)	60.2 ± 5.03	3.11 ± 2.19**	41.8 ± 1.28	6.28 ± 1.49**	25.4 ± 1.16	3.47 ± 0.49**	16.2 ± 0.18	1.53 ± 0.44**
k (h^{-1})	0.95 ± 0.08	2.72 ± 0.96**	0.23 ± 0.02	1.65 ± 0.25**	0.30 ± 0.23	1.47 ± 0.23**	0.27 ± 0.03	1.56 ± 0.79*
$t_{1/2}$ (h)	0.73 ± 0.07	0.27 ± 0.10**	3.05 ± 0.27	0.42 ± 0.06**	2.30 ± 0.17	0.48 ± 0.08**	2.54 ± 0.28	0.51 ± 0.26*

Each value represents mean ± S.D. (n = 5). $^{*}p < 0.05$, $^{**}p < 0.01$.

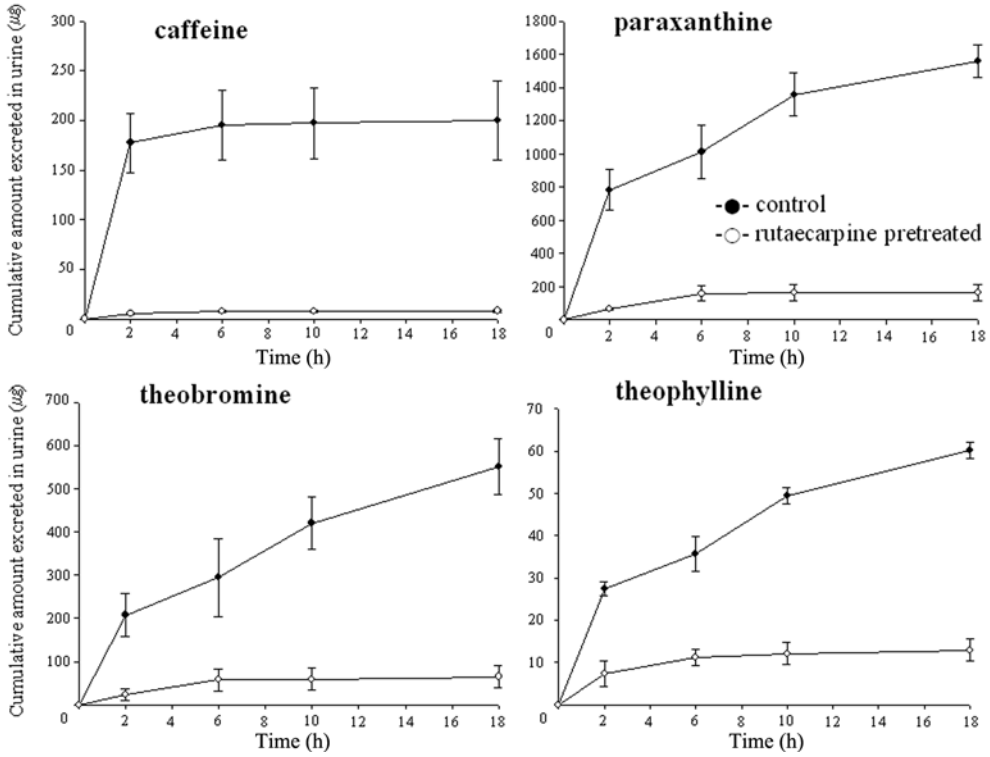

Fig. 2. Cumulative amounts of caffeine, paraxanthine, theobromine, and theophylline excreted in urine following the oral administration of caffeine in the presence and absence of rutaecarpine in rats. Each value represents the mean ± S.D. (n = 5).

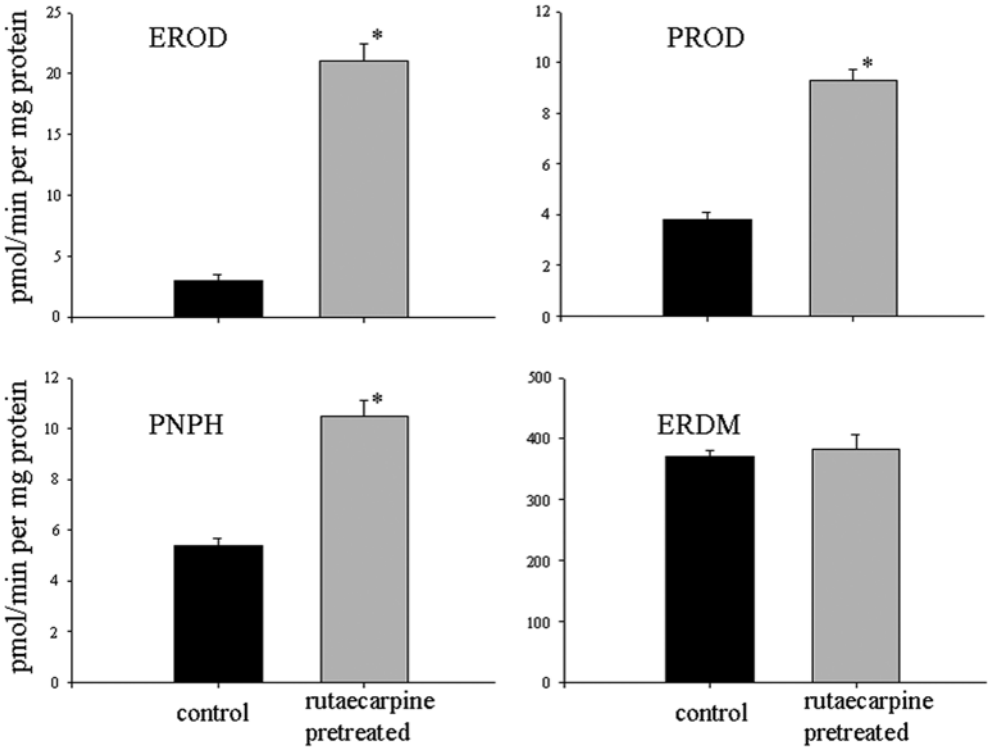

Fig. 3. Effects of rutaecarpine on the cytochrome P450-associated enzymes in the liver S-9 fractions. Each bar represents the mean ± S.D. (n = 5). $^{*}p < 0.01$. EROD, PROD, PNPH and ERDM are abbreviations for ethoxyresorufin *O*-deethylase, pentoxyresorufin *O*-dependentylase, *p*-nitrophenol hydroxylase, and erythromycin N-demethylase, respectively.

bly shortened to 0.5 h. While the C_{max} of the metabolites did not alter, the elimination rates were significantly increased by 5~8 fold; consequently, the AUC_{24h} of paraxanthine, theobromine, and theophylline was only 15, 14, and 9% of the respective value in the control.

The mean cumulative amount of caffeine, paraxanthine, theobromine, and theophylline excreted in urine for 24 h was 200, 1559, 552, and 60 μg, which was equivalent to 3.3, 28.1, 10.0, and 1.1% of the dose, respectively (Fig. 2). In the presence of rutaecarpine, the urinary recovery of those compounds was diminished dramatically to 8, 163, 65, and 13 μg, respectively.

To understand the rutaecarpine-induced changes in the pharmacokinetics of caffeine and its three metabolites, liver S-9 protein content was determined. Fig. 3 shows the CYP enzyme activity in hepatic S-9 fractions isolated from rats pretreated with rutaecarpine for 3 days. The results showed that rutaecarpine significantly increased the relative activities of EROD, PROD, and PNPH, indicating the induction of CYP1A2, 2B, and 2E1 by rutaecarpine, respectively. Rutaecarpine did not change the activity of CYP 3A-specific ERDM.

DISCUSSION

Many drugs were originally derived from herbs and other natural resources. The dried unripe fruit of *Evodia rutaecarpa* has traditionally been used as a remedy for gastrointestinal disorders, headache, amenorrhea, and postpartum hemorrhage (Ueng et al., 2002; Lee et al., 2004a). Rutaecarpine is an excellent example of a biologically active constituent from natural resources, not only because it induces some CYP enzymes, but also because its metabolic pathway, including phases 1 and 2, has clearly been characterized (Lee et al., 2004a, 2004b, 2005; Jan et al., 2005). As shown in previous studies, rutaecarpine induces the activity of hepatic CYPs (Ueng et al., 2001, 2002; Lee et al., 2004a). In particular, rutaecarpine increases CYP 1A and 2E1 levels at 20 mg/kg and 80 mg/kg, respectively (Lee et al., 2004a). At doses of 40 and 80 mg/kg, rutaecarpine also increases the CYP 1A2 activity (Ueng et al., 2001), and ingestion of *Wu-chu-yu-tang*, a herbal preparation containing *E. rutaecarpa*, elevated the mouse hepatic CYP1A2 activity and protein level (Ueng et al., 2002).

Those studies suggested that drug interactions involving rutaecarpine should be expected. However,

there is very limited information on drug-drug interactions (Jan et al., 2005; Ueng et al., 2005; Tsai et al., 2005). Theophylline levels were significantly and dose-dependently decreased by pretreatment with rutaecarpine, an extract of *Evodia rutaecarpa*, or the herbal preparation *Wu-chu-yu-tang* (Jan et al., 2005; Ueng et al., 2005). Recently, we reported that rutaecarpine might also affect the kinetics of acetaminophen and chlorzoxazone in rats by altering their metabolism (Lee et al., 2007; Bista et al., 2008). While Tsai et al. (2005) partially studied the effects of rutaecarpine on plasma caffeine concentrations, in this study, we investigated the influence of the alkaloid on caffeine and its three main metabolites, paraxanthine, theobromine, and theophylline, simultaneously.

When rats were pretreated orally with rutaecarpine, the hepatic CYP1A, 2B, and 2E1 enzymes were increased significantly (Fig. 3). The induction of CYP enzymes may cause drug tolerance or drug-drug-interactions, and our results suggest the pharmacokinetic changes in caffeine and its metabolites due to rutaecarpine.

Caffeine is metabolized by several enzymes: it is demethylated by CYP enzymes, it forms uric acid metabolites via xanthine oxidase; and it is acetylated by N-acetyltransferase (Bechtel et al., 1993; Yuan et al., 2002). All three demethylation reactions are predominantly catalyzed by CYP1A2 and 2E1 (Yuan et al., 2002). The CYP1A2 isoform is exclusively responsible for the 3-N-demethylation of caffeine to form paraxanthine, and CYP2E1 catalyzes the 1-N- and 7-N-demethylation to form theobromine and theophylline, respectively.

In contrast to the general case in which the systemic exposure of metabolites was increased, corresponding to a decrease of that of the parent compound by an enzyme inducer, the plasma concentrations of the three metabolites of caffeine were also decreased on treatment with rutaecarpine. In addition, rutaecarpine remarkably reduced the urinary recovery of caffeine, paraxanthine, theobromine, and theophylline by 96, 89, 88, and 78% of the respective control values.

This seems to occur because the three metabolites are further transformed to hydroxyl- or demethylated-metabolites by CYP1A2 and CYP2E1 (Caubet et al., 2004). This speculation is supported by the shortness of the elimination rate and T_{max} of the metabolites in the presence of rutaecarpine.

Nevertheless, the generation of metabolites was still significantly increased in the presence of rutaecarpine. Namely, the ratio of AUC_{24h} of each metabolite to that of caffeine in the control was 0.69, 0.42, and 0.27 for paraxanthine, theobromine, and theophylline, respectively, and those in the animals pretreated with rutaecarpine were increased to 2.9, 2.6, and 1.8, respectively. In addition, there was a significant increase in the generation rate constant (k_g) extrapolated using a residual method from the elimination phase of each metabolite: the k_g of 1.2 h^{-1} for paraxanthine, 1.4 h^{-1} for theobromine, and 1.1 h^{-1} for theophylline in the controls was increased to 4.8, 5.2, and 4.7 h^{-1} in the presence of rutaecarpine, respectively.

One should also take into account that the decrease in the systemic exposure of caffeine might be attributed to a hindrance in the gastrointestinal (GI) absorption by rutaecarpine. However, so far, no evidence has been found about any effect of rutaecarpine on the transporters in the GI tract, and it could be postulated that the repeated dosing of rutaecarpine might not affect the expression of transporters such as P-gp, because rutaecarpine did not change the activity of CYP 3A-specific ERDM overlapping in substrates with P-gp.

In conclusion, we clearly determined the time course of plasma concentrations and urinary excretion of caffeine and its three major metabolites in the presence and absence of rutaecarpine, and confirmed that rutaecarpine acts as an inducer of hepatic CYP1A2 and 2E1 enzymes, which play major roles in the biotransformation of those compounds.

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