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*-depentylase, 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* (Chiu 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...
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-depentylase (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.