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Effects of Rutaecarpine on the Pharmacokinetics of Caffeine and Its Three Metabolites in Rats

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Abstract

Rutaecarpine, an alkaloid originally isolated from the unripe fruit of *Evodia rutaecarpa*, has been shown to be anti-inflammatory. In the present study, a possible interaction between rutaecarpine and caffeine was investigated in male Sprague Dawley rats. Twenty four hr after the oral pretreatment with rutaecarpine at 80 mg/kg for three consecutive days, rats were treated intravenously with 10 mg/kg of caffeine. Compared with control rats, the pharmacokinetic parameters of caffeine in rutaecarpine-pretreated rats were significantly changed, possibly due to the rapid metabolism. The production of three metabolites of caffeine (i.e., paraxanthine, theobromine and theophylline) was also significantly changed in rats pretreated with rutaecarpine. The present results suggest that oral rutaecarpine would change the intravenous pharmacokinetic characteristics of caffeine.

Key Words: Rutaecarpine, Caffeine, Pharmacokinetics, Intravenous, Rat, *In vivo*

INTRODUCTION

Plant-derived medicines have long been used in Eastern Asia including Korea. As an example, *Evodia rutaecarpa* has been used for the treatment of gastrointestinal disorders, headache and amenorrhea (Ueng *et al.*, 2002a; Lee *et al.*, 2004a). A biological activity of its alkaloid component, rutaecarpine (8,13-dihydro-7H-indolo-[2',3':3,4]-pyrido-[2,1-*b*]-quinazolin-5-one), has also been studied (Chiou *et al.*, 1996) (Fig. 1). Among its activities, rutaecarpine has recently been demonstrated to have anti-inflammatory activity through cyclooxygenase-2 inhibition (Moon *et al.*, 1999; Woo *et al.*, 2001). In addition to the efficacy, rutaecarpine is known to induce CYP1A, CYP2B and CYP2E1 in mice and rats based on measuring enzyme activity (Ueng *et al.*, 2001; Lee *et al.*, 2004a). Although rutaecarpine might interact with other drugs, related studies have been very limited. For example, rutaecarpine has been known to alter pharmacokinetic parameters of theophylline in rats (Ueng *et al.*, 2005).

Along with its wide use in soft drinks, caffeine is also contained in numerous prescription and over-the-counter drugs. It is easily absorbed and rapidly distributed throughout all tissues. Along with its inductive effects on hepatic CYP1A2 in rodents (Chen *et al.*, 1996), caffeine has been known to be metabolized by hepatic CYP1A2 and CYP2E1 (Caubet *et al.*, 2004). Thereby, N-demethylations of caffeine have been used as marker reactions for some CYPs: 3-N-demethylation to paraxanthine catalyzed by CYP1A2 and 1-N-demethylation to theobromine and 7-N-demethylation to theophylline catalyzed by CYP2E1. Furthermore, caffeine and its three major metabolites have also been used as a tool for studying drug-drug interactions. For example, we recently reported that rutaecarpine might cause significant changes in oral caffeine pharmacokinetics (Noh *et al.*, 2011). In the study, it was found that caffeine was rapidly converted to its three N-demethylated metabolites, which were also quickly transformed to further metabolites, when animals were pretreated with rutaecarpine, a CYP inducer. Further metabolism of three demethylated metabolites to others was supported by significant induction of CYP1A2 and CYP2E1 by rutaecarpine (Lee *et al.*, 2004a). Meanwhile, no evidence has been found in the study, regarding possible effects of rutaecarpine on the transporters for caffeine absorption in gastrointestinal tract, and it was postulated that the repeated dose of rutaecarpine might cause the expression of drug transporters in our recent study (Noh *et al.*, 2011).

The purpose of the present study was to investigate the pharmacokinetics of intravenous caffeine with its three metab-

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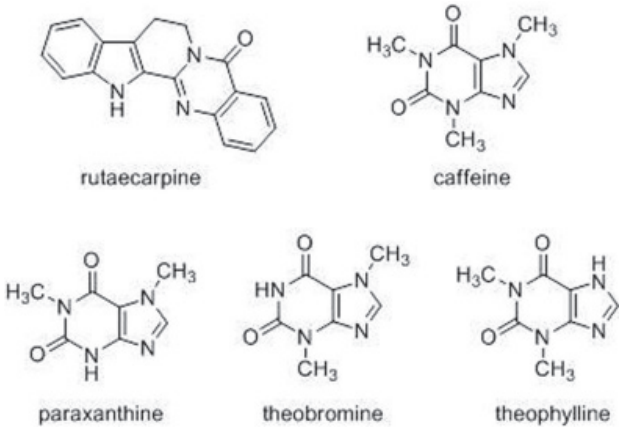

Fig. 1. Chemical structures studied in the study.

olites following oral rutaecarpine pretreatment in rats in order to study whether the transporters might have a role in caffeine pharmacokinetics. Intravenous route of caffeine was selected to rule out the possible role of transporters during absorption in gastrointestinal tract.

MATERIALS AND METHODS

Materials

Rutaecarpine (purity >99%) used in this study was chemically synthesized by our group (Lee *et al.*, 2001). Caffeine and its metabolites, paraxanthine, theobromine and theophylline, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol (MeOH) and acetonitrile (ACN) were HPLC-grade from Merck Ltd. (Poole, UK). All other chemicals were of analytical grade and used as received.

Animals

Specific pathogen-free male Sprague-Dawley rats (250-280 g) were obtained from the Orient Co. (Seoul, Korea). The animals were received at 6 weeks of age and were acclimated for at least one week. Upon arrival, animals were randomized and housed three per cage in strictly controlled conditions of 23 ± 3°C and 50 ± 10% relative humidity. A 12-hr light and 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 (USA).

Animal treatment

Rats were randomly divided into two groups, without (control, n=5) and with (n=5) oral rutaecarpine. Rats received oral rutaecarpine dissolved in corn oil at a dose of 80 mg/kg/day using a stomach tube once a day for three consecutive days, followed by an injection of 10 mg/kg caffeine dissolved in saline via penile vein one day after the last dose of rutaecarpine. Ten ml/kg of corn oil was orally administered to the control rats. A blood sample (200 μl) was collected via the subclavian vein immediately before and 0.033, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 10 hr after the injection of caffeine. The blood samples were centrifuged at 3,000×g for 20 min at 4°C to obtain serum samples. The sera were stored at −80°C until use. No difference was found in the quantitation of analytes between serum and plasma (Data not shown).

LC-tandem mass spectrometry

For sample preparation, 90 μl of internal standard solution (IS, 1 μg thiamphenicol in 1 ml MeOH) was added to 30 μl of serum. After vortex mixing for 10 min and centrifugation at 15,000×g at 15°C to remove the proteins, a 3 μl supernatant was used for the LC-MS analysis. The HPLC consisted of Agilent 1100 system (Agilent Technologies, Palo Alto, CA, USA) with the API 4000 trap mass spectrometer (Sciex Division of MDS, Toronto, Canada) equipped with an electrospray ionization source. The Zorbax SB C_{18} (2.1×150 mm, 2.5 μm) column was used for the separation. Column temperature was maintained constant at 35°C. The HPLC mobile phases were consisted of 100% ACN 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 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, 58, 51 and 61 V; entrance potential, all of 10 V; collision energy, 28, 27 and 25 V; and collision exit potential, 8, 10 and 12 V, respectively. Theophylline and thiamphenicol (IS) were detected by negative ion mode of *m/z* 178.8 → 163.6 and 354.1 → 289.7; declustering potential, −55 and −74 V; entrance potential, all of −10 V; collision energy, −28 and −17 V; and collision exit potential, −9 and −7 V, respectively. Nitrogen was used as the collision gas for the tandem mass spectrometric 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 serum.

Pharmacokinetic analysis

The total area under the mean serum concentration-time curve from time zero to time infinity (AUC_{last}) was calculated using the trapezoidal rule-extrapolation method for caffeine (Chiou, 1978). The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant. Standard methods were used to calculate the following pharmacokinetic parameters using a non-compartmental analysis (WinNonlin; version 2.1; Scientific Consulting Inc., Cary, NC, USA): AUC, elimination rate constant (k_{el}), terminal half-life ($t_{1/2(\beta)}$), time-averaged total body clearance (CL), the mean residence time (MRT_{last}), and apparent volume of distribution at a steady state (V_{ss}). The results obtained were expressed as the mean ± S.E. and the statistical significance of the results was analyzed at $p<0.01$ (*).

RESULTS

Fig. 2 shows the mean serum concentration-time curve of intravenous caffeine following pretreatment of rats with 80 mg/kg of rutaecarpine for three consecutive days. The pharmacokinetic parameters of caffeine are summarized in Table 1. The control group showed that concentration of caffeine in serum was slowly decreased and still detected 10 hr after the injection. The AUC_{last} was 50.84 ± 2.65 μg · hr/ml and the $t_{1/2(\beta)}$ was 2.38 ± 0.55 hr. Meanwhile, the results from the pretreatment

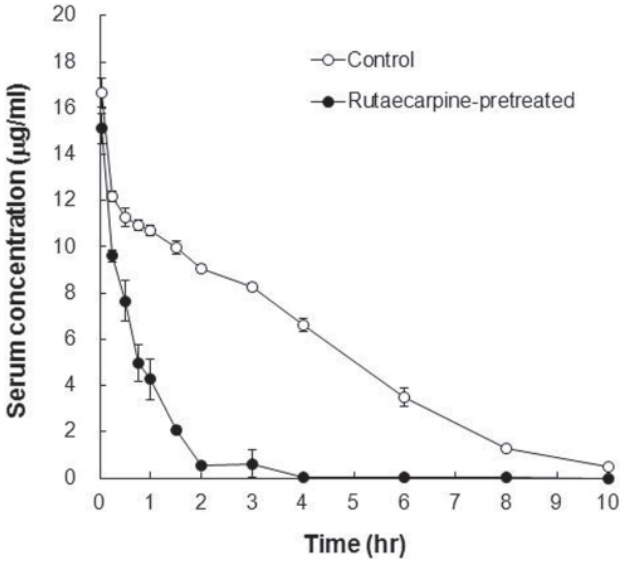

Fig. 2. Mean serum concentration of caffeine in rats after intravenous injection. Rats pretreated orally with 80 mg/kg rutaecarpine in corn oil for 3 days were intravenously treated with 10 mg/kg caffeine in saline one day after the last dose of rutaecarpine. Blood was obtained from the subclavian vein at various time intervals described in the Materials and Methods. Each value represents the mean ± S.E. of five animals.

Table 1. Pharmacokinetic parameters of caffeine in rats after intravenous injection

Parameter	Control (n=5)	With rutaecarpine (n=5)
AUC_{last} (μg · hr/ml)	50.84 ± 2.65	10.67 ± 0.92*
k_{el} (hr^{-1})	0.35 ± 0.05	1.77 ± 0.20*
$t_{1/2(\beta)}$ (hr)	2.38 ± 0.55	0.42 ± 0.06*
CL (L/hr/kg)	0.05 ± 0.00	0.26 ± 0.02*
MRT_{last} (hr)	2.66 ± 0.20	0.71 ± 0.05*
V_{ss} (L/kg)	0.17 ± 0.01	0.21 ± 0.03

Each value represents mean ± S.E. of five animals. The asterisks indicate the values significantly different from the control at $p<0.01$ (*).

group showed that the concentration of caffeine in serum was rapidly decreased and disappeared within 2 hr after caffeine injection. The AUC_{last} was 10.67 ± 0.92 μg · hr/ml and the $t_{1/2(\beta)}$ was 0.42 ± 0.06 hr, which were 21.0% and 17.6% of control, respectively. In our previous study, oral caffeine showed decrease to 5.2% and 37.0% in the AUC and half-life by rutaecarpine pretreatment, respectively (Noh *et al.*, 2011). In addition, MRT_{last} of caffeine significantly decreased to 26.7% of control by rutaecarpine pretreatment. Furthermore, the CL and k_{el} of caffeine were significantly increased by rutaecarpine to 520% and 506% of control, respectively. These results indicate that rutaecarpine might cause fast metabolism of caffeine to its metabolites. Subsequently, therefore, three major metabolites of caffeine were analyzed in rat sera. Three metabolites were analyzed only up to 10 hr in the present study, because our recent study showed rapid production of these metabolites

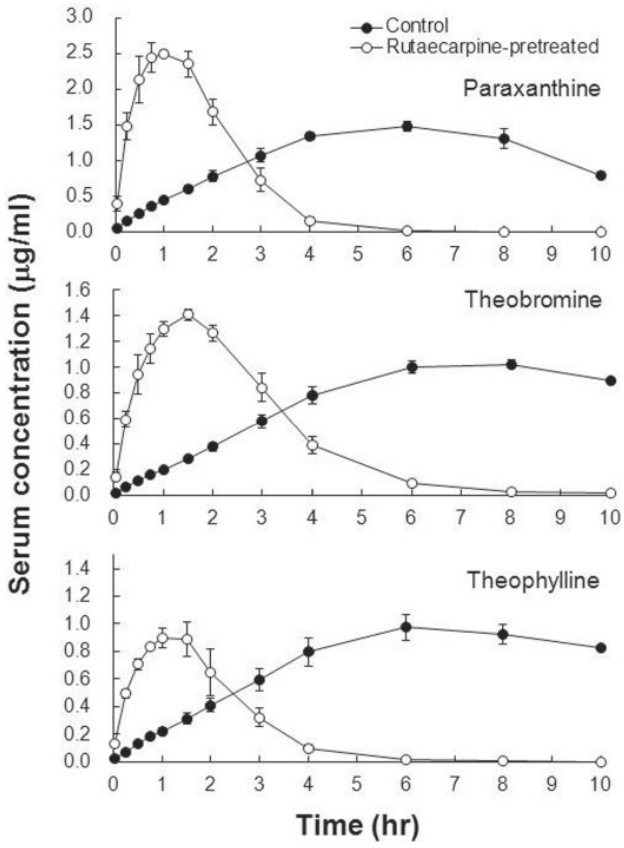

Fig. 3. Mean serum concentration of paraxanthine, theobromine and theophylline in rats after intravenous injection of caffeine. Each value represents the mean ± S.E. of five animals. The experimental conditions were the same as in Fig. 2.

(Noh *et al.*, 2011).

Fig. 3 shows the mean serum concentration-time curve of three major metabolites produced from caffeine following the pretreatment of rats with rutaecarpine. Paraxanthine, a metabolite by CYP1A2, was produced gradually until 10 hr after caffeine treatment, showing maximum production at 6 hr after treatment in control rats. Meanwhile, paraxanthine production in rutaecarpine-pretreated rats showed T_{max} at 1 hr after treatment and disappeared within 6 hr in sera. The results indicate that rutaecarpine pretreatment could also cause dramatic changes in the pharmacokinetics of caffeine metabolites. Furthermore, similar results were obtained in the production of theobromine and theophylline, metabolites produced by CYP2E1. Taken together, the results indicate that production of paraxanthine, theobromine and theophylline from caffeine could also be changed by rutaecarpine, and that the results were very similar with the metabolite production pattern following oral caffeine (Noh *et al.*, 2011).

DISCUSSION

Many drugs were originally derived from herbs and other natural resources. In many regions of the world, herbal medicines have been used for treating a variety of diseases for thousands of years. Many herbal preparations are claimed to

be effective in treating diseases, but, in most cases, the active ingredient(s) in these herbal mixtures are unknown and the mechanisms of action are obscure. For this reason, many researchers enthusiastically work in the fields of the identification of new constituents with biological activities, the total or semi-synthesis of active constituents, and characterization of their metabolic profiles *in vitro* and *in vivo*. Rutaecarpine is an example of biologically active constituents with anti-inflammatory activity from natural resources, not only because it could induce some CYP enzymes, but also because its metabolic pathways including phase 1 and phase 2 have clearly been characterized (Lee *et al.*, 2004a; 2004b; 2005; 2006). Meanwhile, extensive attention on possible herb-drug interaction might have not been given for this compound.

As aforementioned in the Introduction, information on drug-drug interactions has been very limited, although series of studies with rutaecarpine implicated possible interactions (Jan *et al.*, 2005; Tsai *et al.*, 2005; Ueng *et al.*, 2005). In the previous studies, theophylline level was significantly and dose-dependently decreased by pretreatment with either rutaecarpine, extract of *Evodia rutaecarpa* or the herbal preparation *Wu-chu-yu-tang* in which the extract of *Evodia rutaecarpa* is included (Jan *et al.*, 2005; Ueng *et al.*, 2005). Our recent studies also showed that pretreatment of rats with rutaecarpine also significantly decreased in the serum levels of acetaminophen and chlorzoxazone when compared with controls (Lee *et al.*, 2007; Bista *et al.*, 2008). Most recently, we found that induction of hepatic CYP1A2 and CYP2E1 by rutaecarpine might play major roles in biotransformation of caffeine from the study to determine the time course of plasma concentrations and urinary excretion of caffeine and its three major metabolites in rutaecarpine-pretreated rats followed by oral caffeine (Noh *et al.*, 2011). Therefore, inductive effects of rutaecarpine on several CYP enzymes seem to be obvious *in vivo*, although it might inhibit some CYP enzymes in liver microsomes *in vitro* (Ueng *et al.*, 2002b).

During the oral study with caffeine, possible role of transporter in the absorption process in gastrointestinal tract could not be ruled out. Therefore, intravenous pharmacokinetic study of caffeine was designed and performed in the present study to compare the results with our previous oral study. In the present study, the results similar with those of previous studies were observed (Noh *et al.*, 2011). When rats were treated with caffeine intravenously after rutaecarpine pretreatment for three days, some pharmacokinetic parameters of caffeine were significantly changed as seen in the oral caffeine study. Compared with control rats, rutaecarpine pretreatment caused 79.0% decrease in the AUC of caffeine, and this could have been due to significantly faster CL which showed a 520% increase (Table 1). The faster CL was, at least in part, attributable to the induction of CYP1A2 and 2E1 by rutaecarpine as observed in our previous study (Noh *et al.*, 2011). In addition, the MRT_{last} and $t_{1/2(\beta)}$ of caffeine in rats with rutaecarpine pretreatment became shorter (i.e., 73.3% and 82.4% decrease) than those in control rats, respectively.In other words, when rats were treated with caffeine intravenously after rutaecarpine pretreatment, the same pharmacokinetic parameters of caffeine changed were also changed similarly in the previous oral study. From the results, it could be concluded that the role of transporters during gastrointestinal absorption might be negligible, although the effect of rutaecarpine pretreatment on the expression of transporters were not investigated in the gastrointestinal tract.Meanwhile, because the increased production of more metabolites might not be the only reason for changing the caffeine pharmacokinetics, effects of rutaecarpine on the distribution of caffeine and the expression of other enzymes possibly involved in the metabolism of caffeine should be considered further. In addition, effects of rutaecarpine on expression of some transporters in the gastrointestinal tract are currently under investigation to confirm our hypothesis.

In addition to the pharmacokinetic changes of caffeine by rutaecarpine pretreatment, rutaecarpine also affected the formation of paraxanthine, theobromine and theophylline from intravenous caffeine, as seen in the oral study (Fig. 3). These results indicate that rutaecarpine might increase in the biotransformation of caffeine to its metabolites much faster and that three metabolites formed might be further metabolized to other metabolites by some enzymes induced by rutaecarpine pretreatment. Reduced urinary excretion of these three metabolites in the previous oral study strongly supports the idea of further metabolism (Noh *et al.*, 2011). In fact, caffeine would be metabolized by CYP enzymes (i.e., N-demethylation), xanthine oxidase (i.e., formation of uric acid metabolites) and/or N-acetyltransferase (i.e., N-acetylation)(Bechtel *et al.*, 1993; Yuan *et al.*, 2002). Among these, three demethylation reactions are predominantly catalyzed by CYP1A2 and 2E1 (Yuan *et al.*, 2002). In addition, these demethylated metabolites have been known to be further metabolized to hydroxyl or demethylated metabolites by CYP1A2 and CYP2E1 (Caubet *et al.*, 2004). Decreases in many parameters including elimination rate constant also support the further metabolism of these three metabolites (Table 1). Meanwhile, possible effects of rutaecarpine on other enzymes, such as xanthine oxidase and N-acetyltransferase should be studied further in the future.

When rats were treated orally with rutaecarpine, hepatic CYP1A, CYP2B and CYP2E1 enzymes were significantly increased in our previous studies (Lee *et al.*, 2004a; Noh *et al.*, 2011). The induction of CYP enzymes would cause drug tolerance and/or drug-drug interactions. In particular, CYP3A enzymes are very important, because CYP3A is a subfamily of the most abundant drug-metabolizing CYP enzymes, and because many drugs currently available are predominantly metabolized by CYP3A enzymes. Fortunately, however, the induction of CYP3A proteins was not observed during pretreatment with rutaecarpine (Lee *et al.*, 2004a; Noh *et al.*, 2011).

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