

## Effect of Phenobarbital on the Pharmacokinetics of Rutaecarpine and its Metabolite in Rats

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**Abstract** – To investigate the possible interaction between rutaecarpine and phenobarbital in rats, phenobarbital in saline at 80 mg/kg was given ip to male SD rats for 3 consecutive days. Saline was given to control animals. One day after phenobarbital pre-treatment, rutaecarpine at 16 mg/kg was administered through penile vein. Blood was collected and analyzed by using HPLC. The pharmacokinetic parameters were determined with the non-compartmental model. Pre-treatment with phenobarbital significantly altered the pharmacokinetic profiles of rutaecarpine and its metabolite, 10-hydroxyrutaecarpine. The AUC of rutaecarpine was reduced to approximately 50% of control and the plasma half-life of rutaecarpine was significantly shortened when compared with control. In addition, the C<sub>max</sub> of 10-hydroxyrutaecarpine was increased approximately 160% of control. The AUC and the plasma half-life of 10-hydroxyrutaecarpine were decreased to 76.9% of control and to 82.7 min from 175.9 min, respectively. The results suggested that phenobarbital might accelerate the metabolism of rutaecarpine, thereby changing the pharmacokinetic parameters of rutaecarpine in male SD rats.

**Key words:** Rutaecarpine, Phenobarbital, Drug interaction, Pharmacokinetics, Metabolite, *in vivo*

### INTRODUCTION

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). In addition to its traditional use in treatment of gastrointestinal disorders, rutaecarpine has recently been characterized to have an anti-inflammatory activity through cyclooxygenase-2 inhibition (Moon *et al.*, 1999; Woo *et al.*, 2001). More recently, in order to develop rutaecarpine as an anti-inflammatory agent, a simple and practical method for the synthesis of rutaecarpine in a large quantity was successfully established by our group (Lee *et al.*, 2001). Previous studies reported that cytochrome P450 (CYP) 1A and 2B might predominantly metabolize rutaecarpine in rat liver microsomes (Lee *et al.*, 2004a) and that nine phase I metabolites of rutaecarpine were identified by using liquid chromatography-electrospray ionization tandem mass spectrometry (Lee

*et al.*, 2004b).

Phenobarbital (PB), one of the antiepileptic drugs, induces drug-metabolizing enzymes in laboratory animals and human, including CYP 2B and 3A (Park *et al.*, 2005). PB has been widely used as a prototype inducer for drug metabolism in pharmacological and toxicological investigations.

Although many constituents isolated from natural resources have been investigated for the development of new drug candidates, the possible herb-drug interactions were hardly studied. Particularly, the *in vivo* interaction of rutaecarpine with CYP inducers and inhibitors has not been investigated to date. To develop rutaecarpine as an anti-inflammatory drug, our group has focused our research on its metabolism (Lee *et al.*, 2004a, Lee *et al.*, 2005, Park *et al.*, 2005) and, for this reason, it was necessarily required to study the possible interaction of rutaecarpine with a well known CYP inducer, PB.

In the present study, the pharmacokinetic parameters of rutaecarpine and its metabolite were investigated in rats following pre-treatment with phenobarbital as a part of new drug development.

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## MATERIALS AND METHODS

### Materials

Rutaecarpine (purity, >99%) used in this study was chemically synthesized in our group (Lee *et al.*, 2001). Tryptanthrin (purity, >99.8%) was obtained by the chemical synthesis described previously (Son *et al.*, 2003). PB was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade and all solvents were of HPLC grade.

### Animal studies

Specific pathogen-free male Sprague Dawley rats (250–280 g) were obtained from the Orient Co. (Seoul, Korea). The animals received at 6 weeks of age were acclimated for at least 1 week. Upon arrival, animals were randomized and housed 3 per cage. The animal quarters were strictly maintained at  $23 \pm 3^\circ\text{C}$  and  $50 \pm 10\%$  relative humidity. A 12 h light and dark cycle was used with an intensity of 150–300 Lux. All animal procedures were followed based on a guideline recommended by the Society of Toxicology (USA) in 1989. PB in saline at 80 mg/kg was given ip to rats for 3 consecutive days. For control, saline was given to rats at 10 ml/kg. 24 h after the last dosing, rutaecarpine in 1% povidone solution at 16 mg/kg was administered into penile vein. Approximately 300  $\mu\text{l}$  of blood samples were collected from the subclavian vein immediately before and 5, 10, 20, 40, 60, 120, 180, and 240 min after the rutaecarpine administration. Blood samples were centrifuged at 3000 $\times$ g for 15 min at  $4^\circ\text{C}$  to obtain sera.

### HPLC analysis

5  $\mu\text{l}$  of internal standard (IS; 5  $\mu\text{g}/\text{ml}$  of tryptanthrin) was added to serum samples and then 1 ml of ethyl acetate was added to deproteinize serum samples. Following centrifugation at 3,000 $\times$ g for 10 min at  $15^\circ\text{C}$ , the supernatant (0.85 ml) was completely evaporated with a stream of nitrogen gas. The residue was reconstituted with 100  $\mu\text{l}$  of methanol and centrifuged at 10,000 $\times$ g for 10 min. 20  $\mu\text{l}$  aliquot was injected into the HPLC system.

The HPLC system consisted of a pump (LC-20AD), an autoinjector (SIL-20A), a UV-Visible detector (SPD-20A), and a communications bus module (CBM-20A) from Shimadzu Scientific Instruments (Kyoto, Japan). A Waters COSMOSIL  $5\text{C}_{18}$ -MS-II column (5  $\mu\text{m}$ , 4.6  $\times$  250 mm) was used for the HPLC separation.

Separation was conducted using a gradient solvent system from 40:60 to 80:20 for 35 min with 100% of acetonitrile:20 mM ammonium formate buffer, pH 6.8, at a

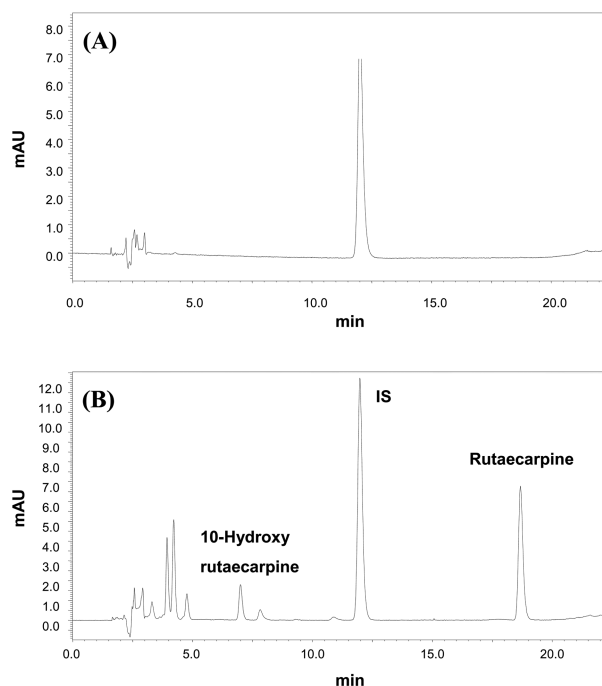
flow rate of 1.2 ml/min. The eluate was monitored at 344 nm. The data was acquired as the ratio of the AUC of samples with AUC of IS. The spiked concentration of the calibration standard was linear over the concentration range of 0.05 mg/ml to 3 mg/ml.

### Pharmacokinetic parameters and statistics

The pharmacokinetic parameters were determined using the standard non-compartmental method. Serum AUC was calculated using WinNonlin (version 1.1, Scientific Consulting, KY, USA) with a log linear trapezoidal method. The results were expressed as mean  $\pm$  S.E. and the significance of data was analyzed with Student's *t*-test. The values significantly different from control were expressed as asterisks at either  $P < 0.05$  (\*) or  $P < 0.01$  (\*\*).

## RESULTS

Representative UV chromatograms of blank serum and serum isolated from rats treated intravenously with rutaecarpine are shown in Figure 1. Rutaecarpine, IS, and 10-hydroxyrutaecarpine were detected at the retention time of



**Fig. 1.** Chromatograms of the blank serum (A) and the serum treated intravenously with 16 mg/kg rutaecarpine for 10 min (B). Rutaecarpine, internal standard (IS; tryptanthrin) and 10-hydroxyrutaecarpine were detected at the retention times of 18.6, 11.9, and 6.9 min, respectively. The concentration of IS, tryptanthrin, was 5  $\mu\text{g}/\text{ml}$ .

**Table I.** Pharmacokinetic parameters of rutaecarpine and its metabolite following phenobarbital pre-treatment in rats

Parameters	Rutaecarpine		Parameters	10-Hydroxyrutaecarpine	
	Control	Phenobarbital pre-treated		Control	Phenobarbital pre-treated
-	-	-	$T_{max}$ (min)	26.7±6.7	10.0±0.0
-	-	-	$C_{max}$ (%)	100.0±0.0	160.0±27.4*
$AUC_{\infty}$ (µg·min/ml)	115.7±8.8	64.0±0.3*	AUC (%)	100.0±0.0	76.9±2.6*
$T_{1/2}$ (min)	56.7±1.2	35.7±0.8**	$T_{1/2}$ (min)	175.9±2.7	82.7±10.1**

Phenobarbital in saline at 80 mg/kg was given ip to male SD rats for 3 consecutive days. For control, saline was given to rats at 10 ml/kg. 24 h after the last dosing, rutaecarpine in 1% povidone solution at 16 mg/kg was administered through penile vein. Blood samples were collected from the subclavian vein immediately before and 5, 10, 20, 40, 60, 120, 180, and 240 min after the rutaecarpine administration. Each value represents mean ± S.E. of three animals. The asterisks indicate the values significantly different from control at either  $P < 0.05$  (\*) or  $P < 0.01$  (\*\*).

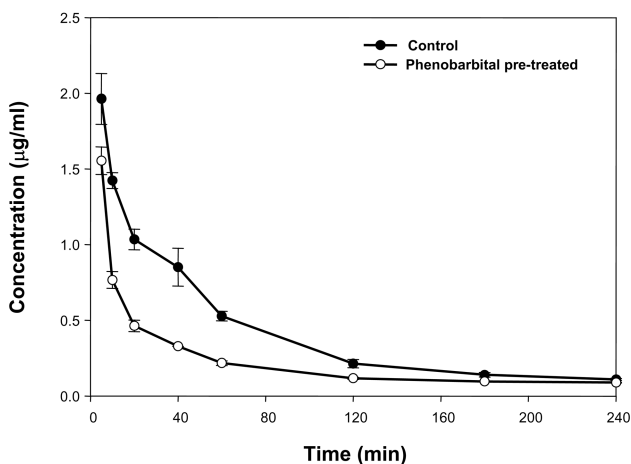
18.6, 11.9, and 6.9 min, respectively. 10-Hydroxyrutaecarpine was identified and confirmed by using liquid chromatography-electrospray ionization tandem mass spectrometry and by previous literatures (Lee *et al.*, 2005, Ueng *et al.*, 2005).

The mean serum concentration-time curve of rutaecarpine and 10-hydroxyrutaecarpine are shown in Figure 2 and Figure 3, respectively. As shown in Table I, the pharmacokinetic parameters of the group pre-treated with PB were significantly changed when compared with the control where rutaecarpine was administered alone. The AUC and the terminal plasma half-life ( $T_{1/2}$ ) of rutaecarpine decreased significantly in the group pre-treated with PB.

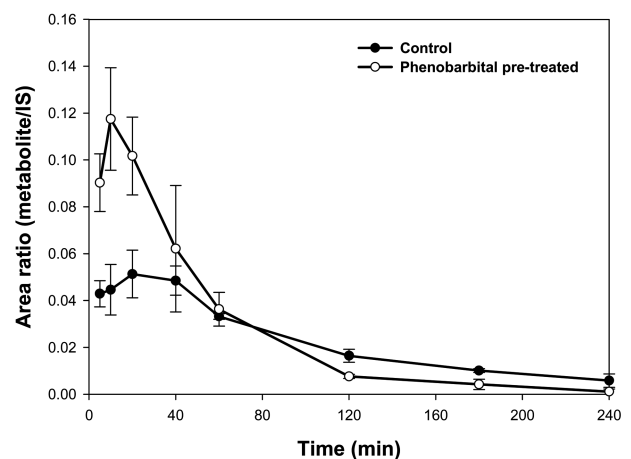
Although the authentic standard of 10-hydroxyrutaecarpine was not available, some pharmacokinetic parameters could be deduced in the present study. When rats were pre-treated with PB, the  $C_{max}$  of 10-hydroxyrutaecarpine was increased to 1.6-fold of control and the  $T_{max}$  reached approximately 2.7-fold faster than control group. In addition, PB pretreatment significantly decreased the AUC of metabolite to 76.9% of control, and the terminal plasma half-life ( $T_{1/2}$ ) to 82.7 min from 175.9 min of control.

## DISCUSSION

There are many reasons causing failure in new drug



**Fig. 2.** Mean serum concentration-time profile of rutaecarpine in male SD rats following an intravenous injection with 16 mg/kg rutaecarpine. Blood was collected from the subclavian vein immediately before and 5, 10, 20, 40, 60, 120, 180, and 240 min after the dose. Each value represents the mean±S.E. of three animals.



**Fig. 3.** Mean serum area ratio for metabolites/internal standard (IS)-time profile of 10-hydroxyrutaecarpine in male SD rats following an intravenous injection with 16 mg/kg rutaecarpine. Blood was collected from the subclavian vein immediately before and 5, 10, 20, 40, 60, 120, 180, and 240 min after the dose. Each value represents the mean±S.E. of three animals.

development, such as inappropriateness of drug metabolic or pharmacokinetic characteristics including decreased pharmacological effect in *in vivo* system, toxicity, very short half-life, and induction of specific enzymes. To overcome or avoid these problems, the importance of studies on drug metabolism and pharmacokinetics is emphasized in the early stage of drug development nowadays. In addition, studying pharmacokinetic profiles of parent compound together with their metabolites can play a very useful role in new drug development to prevent possible financial damage that might be occurred from the failure in a late stage. For these reasons, the possible interaction of rutaecarpine with PB was investigated in the present study. PB was selected as a CYP inducer, because our previous report indicated that microsomes isolated from PB-pretreated rats could significantly increase the metabolism of rutaecarpine *in vitro* (Lee *et al.*, 2004a).

As mentioned in the Introduction, rutaecarpine has beneficial pharmacological activities including anti-inflammatory activities (Moon *et al.*, 1999, Woo *et al.*, 2001). In addition, we have recently identified 9 phase I metabolites with their chemical structures by using tandem mass spectrometry (Lee *et al.*, 2004b). Therefore, rutaecarpine would be a good model compound to study the drug-drug interaction, because one can study the possible kinetic changes in metabolite production together with the parent drug.

In the present LC conditions, however, 10-hydroxyrutaecarpine was only detected among 9 phase I metabolites in rats. Nevertheless, we could deduce some pharmacokinetic changes of rutaecarpine and its 10-hydroxy metabolite following PB pretreatment. Pretreatment of rats with PB significantly reduced the plasma level of rutaecarpine with its half-life. In addition, PB pretreatment significantly changed the  $T_{max}$  of 10-hydroxy metabolite. These results clearly indicated that the induction of CYP by PB significantly increased in the metabolism of rutaecarpine to its phase I metabolite. The present results were consistent with our previous reports that the metabolism of rutaecarpine is CYP dependent in rat liver microsomes (Lee *et al.*, 2004a, Lee *et al.*, 2004b). Although only one metabolite was detected in the present study, it was evident that PB can modulate the metabolism of rutaecarpine in rats. It

would be more informative when the pharmacokinetic parameters for all phase I metabolites are available.

## ACKNOWLEDGEMENT

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