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 cyclo-oxygenase-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-
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, version 2.1; Scientific Consulting Inc., Cary, NC, USA: AUC, elimination rate constant (k_e), terminal half-life (t_1/2β), time-averaged total body clearance (CL), the mean residence time (MRT), and apparent volume of distribution at a steady state (Vss). 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 AUCbio was 50.84 ± 2.65 μg·hr/ml and the t1/2β was 2.38 ± 0.55 hr. Meanwhile, the results from the pretreatment
group showed that the concentration of caffeine in serum was rapidly decreased and disappeared within 2 hr after caffeine injection. The AUC\textsubscript{last} was 10.67 ± 0.92 μg · hr/ml and the t\textsubscript{1/2}(β) 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). Furthermore, the CL and k\textsubscript{e} 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 (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 acetamiphen 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 MRTlast and t1/2 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).

ACKNOWLEDGMENTS

This work was supported by a grant from Yeungnam University in 2009 (209-A-380-160).

REFERENCES


