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Enhanced oral exposure of diltiazem by the concomitant use of naringin in rats

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Abstract

The present study aims to investigate the effect of naringin, a flavonoid, on the pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem, in rats. Pharmacokinetic parameters of diltiazem and desacetyldiltiazem were determined in rats following an oral administration of diltiazem (15 mg kg^{-1}) to rats in the presence and absence of naringin (5 and 15 mg kg^{-1}). Compared to the control given diltiazem alone, the C_{max} and AUC of diltiazem increased by twofolds in rats pretreated with naringin, while there was no significant change in T_{max} and terminal plasma half-life ($T_{1/2}$) of diltiazem. Consequently, absolute and relative bioavailability values of diltiazem in the presence of naringin were significantly higher ($p < 0.05$) than those from the control group. Metabolite–parent AUC ratio in the presence of naringin decreased by 30% compared to the control group, implying that naringin could be effective to inhibit the metabolism of diltiazem. In conclusion, the concomitant use of naringin significantly enhanced the oral exposure of diltiazem in rats.

Keywords: Diltiazem; Desacetyldiltiazem; Pharmacokinetics; Naringin; Rat

1. Introduction

With the great interest in herbal products as alternative medicines, herbal products are ingested by about 10% or more of the general population and 30–70% of individuals with specific disease states (Duggan et al., 2001; Ni et al., 2002). Accordingly, there is growing interest in the potential drug interactions between herbal products and the conventional drugs, which may alter the efficacy or adverse effect of drugs, however, there is far less information on the pharmacokinetic interactions between herbal products and medicines. Therefore, more preclinical and clinical investigations on the herbal constituents–drug interaction should be performed to prevent potential adverse reactions or to utilize those interactions for a therapeutic benefit. Recently, increasing in vitro and in vivo evidences have indicated that the pharmacokinetic interaction of drugs with herbal products may be attributable to the modulation of metabolizing

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enzymes, and drug transporters such as P-gp (Evans, 2000; Ioannides, 2002; Zhou et al., 2004). Many herbal constituents, in particular, flavonoids, were reported to modulate P-gp by interacting directly with the vicinal ATP-binding site, or the substrate-binding site (Zhang and Morris, 2003; Zhou et al., 2004). In addition, flavonoids appear to be effective to modulate the hepatic- and intestinal-metabolizing enzymes (in particular, CYP3A4) (Miniscalco et al., 1992; Hodek et al., 2002).

Among flavonoids, naringin (4′,5,7-trihydroxy-flavanone-7-rhamnoglucoside) is the predominant component in grapefruit juice and transformed into naringenin by the bacteria in human intestinal microflora (Ameer and Weintraus, 1997; Kim et al., 1998). Both naringin and naringenin reduced apical efflux of vinblastine, a substrate of P-gp in Caco-2 cells (Takanaga et al., 1998). In addition, naringin and naringenin also inhibit CYP3A4-mediated saquinavir metabolism and also modulate P-gp transport in Caco-2 cell (Eagling et al., 1999). Therefore, naringin might act as a dual inhibitor of CYP3A4 and P-gp. Several studies have demonstrated that grapefruit juice could alter the pharmacokinetics of certain drugs including felodipine, nicardipine and verapamil and suggested that the drug interaction with grapefruit juice may be caused by a combined inhibition of presystemic metabolism and P-gp during the intestinal drug absorption (Bailey et al., 1998a; Kane and Lipsky, 2000; Zhang and Benet, 2001). However, the components in grapefruit juice responsible for such an interaction have not been fully determined, although particular interest has been focused on the grapefruit flavonoid, naringin, and the furanocoumarin, 6′,7′-dihydroxybergamottin (Fuhr and Kummert, 1995; Bailey et al., 1998b; Ho et al., 2000).

Drug with low oral bioavailability due to presystemic metabolism can be more affected by CYP inhibitors than the drugs with high oral bioavailability. Diltiazem, a calcium channel blocker, is the drug with low bioavailability and high clearance due to the extensive presystemic metabolism as well as P-gp efflux (Yeung et al., 1990; Lee et al., 1991). Therefore, the present study selected diltiazem and evaluated the effect of naringin on the pharmacokinetics of diltiazem in rats to examine a potential drug interaction between naringin and diltiazem via the dual inhibition of metabolic enzymes and P-gp.

2. Materials and methods

2.1. Materials

Diltiazem hydrochloride, desacetyldiltiazem, imipramine hydrochloride and naringin (4′,5,7-trihydroxy-flavanone-7-rhamnoglucoside) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, methanol, *tert*-butylmethylether were obtained from Merck Co. (Darmstadt, Germany). All other chemicals were reagent grade and all solvents were HPLC grade.

2.2. Animal studies

All animal studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) and the experimental protocols were approved by the Animal Care Committee of Chosun University. Male Sprague-Dawley rats (270–300 g) were purchased from Dae Han Laboratory Animal Research and Co. (Choongbuk, Korea), and had free access to normal standard chow diet (Jae and Chow, Korea) and tap water. Animals were kept in these facilities for at least 1 week before the experiment and fasted for 24 h prior to the experiments. Drug solution was prepared by adding diltiazem (5 or 15 mg kg^{-1}) to the distilled water (1 mL). In the previous study reported by Yeung et al. (1990), the oral administration of diltiazem to rats at 15 mg kg^{-1} achieved the plasma level comparable to the therapeutic concentrations in humans. Therefore, in the present study, rats ($n = 6$ per each treatment) were given orally a 15 mg kg^{-1} of diltiazem with either (i) naringin (5 or 15 mg kg^{-1}) 30 min prior to diltiazem administration or (ii) no concomitant treatment (diltiazem alone). Previous studies have reported that pretreatment of naringin appeared to be effective to alter the pharmacokinetics of drugs that are substrates of P-gp and/or CYP3A4 over the dose range of 3–30 mg kg^{-1} in rats (Zhang et al., 2000; Tsai et al., 2001; Choi and Shin, 2005). Therefore, 5 or 15 mg kg^{-1} of naringin was administered to rats in the present study. Separately, a 5 mg kg^{-1} of diltiazem was administered intravenously to rats. Blood samples were collected from the femoral artery at 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h postdose. Blood samples were centrifuged and the plasma

was removed and stored at −40 °C until analyzed by HPLC.

2.3. HPLC assay

The plasma concentrations of diltiazem were determined by the HPLC assay modified from the method of Goebel and Kolle (1985). Briefly, 50 μL of imipramine (2 μg mL^{-1}), as the internal standard, and 1.2 mL of *tert*-butylmethylether were added to 0.2 mL of the plasma samples. The mixture was then stirred for 2 min and centrifuged for 10 min. One milliliter of the organic layer was transferred to a clean test tube and 0.2 mL of 0.01N hydrochloride was added and mixed for 2 min. Fifty microliters of the water layer were injected into the HPLC system. The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu Co., Japan), a UV–vis detector (Model SPD-10A), a system controller (Model SCL-10A), degasser (Model DGU-12A) and an autoinjector (SIL-10AD). The UV detector was set to 237 nm. The stationary phase was a μ-bondapack C_{18} column (3.9 × 300 mm, 10 μm, Waters Co., Ireland) and the mobile phase was methanol:acetonitrile:0.04 M ammonium bromide:triethylamine (24:31:45:0.1, v/v/v/v, pH 7.4, adjusted with acetic acid). The retention times at a flow rate of 1.5 mL/min are as follows: internal standard at 10.5 min, diltiazem at 8.0 min and desacetyldiltiazem at 6.5 min. The calibration curves of diltiazem and desacetyldiltiazem were linear within the range of 10–400 ng mL^{-1}. The intra-day ($n = 5$) and inter-day ($n = 5$) coefficients of variation were less than 5% for diltiazem and desacetyldiltiazem, and 1.5% for imipramine. Recovery (%) assessed from the replicate analysis ($n = 5$) for 5 days by adding 20 and 200 ng mL^{-1} of diltiazem to the rat plasma was shown 106 ± 5.7 and 101 ± 4.9, respectively. Detection limit of diltiazem and desacetyldiltiazem was 10 ng mL^{-1}.

2.4. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed by using Kinetica-4.3 (InnaPhase Corp., Philadelphia, PA, USA). The area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal method. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were observed values from the experimental data. The elimination rate constant (K_{el}) was estimated by regression analysis from the slope of the line of best fit, and the half-life ($T_{1/2}$) of the drug was obtained by $0.693/K_{el}$. The absolute bioavailability (A.B.%) of diltiazem was calculated by $AUC_{oral}/AUC_{i.v.} \times Dose_{i.v.}/Dose_{oral} \times 100$, and the relative bioavailability (R.B.%) of diltiazem was estimated by $AUC_{diltiazem\ w/naringin}/AUC_{control} \times 100$. The metabolite–parent ratio (M.R.) was estimated by $(AUC_{desacetyldiltiazem}/AUC_{diltiazem}) \times (M.W._{diltiazem}/M.W._{desacetyldiltiazem})$.

2.5. Statistical analysis

All the means are presented with their standard deviation. The pharmacokinetic parameters were compared with a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett correction. A p value <0.05 was considered statistically significant.

3. Results and discussion

The mean plasma concentration–time profiles of diltiazem in the presence and absence of naringin were characterized in rats and illustrated in Fig. 1. The mean pharmacokinetic parameters of diltiazem were also summarized in Table 1.

As shown in Table 1, the pharmacokinetic parameters of diltiazem following an intravenous or oral administration of diltiazem were comparable to those from the previous studies (Yeung et al., 1990; Lee et al., 1991). However, the pretreatment of naringin (5 or 15 mg kg^{-1}) prior to diltiazem administration significantly altered the pharmacokinetic profiles of diltiazem compared to the control given diltiazem alone. Presence of naringin significantly enhanced K_a (absorption rate constant) of diltiazem, which might be due to the inhibition of P-gp and intestinal metabolism by naringin (Takanaga et al., 1998; Eagling et al., 1999). The C_{max} and AUC of diltiazem increased by twofolds in the rats pretreated with naringin, while there was no significant change in T_{max} and terminal plasma half-life ($T_{1/2}$) of diltiazem in the presence of naringin (Table 1). Consequently, the absolute and relative bioavailability values of diltiazem in the rats pretreated with naringin (5 or 15 mg kg^{-1}) were significantly higher ($p < 0.05$) than those from the control

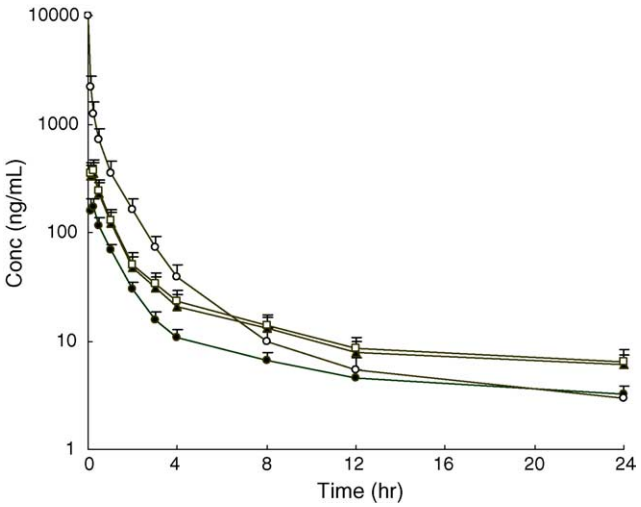

Fig. 1. Mean plasma concentration–time profiles of diltiazem following an intravenous (5 mg kg^{-1}) or oral (15 mg kg^{-1}) administration of diltiazem to rats in the presence and absence of naringin (mean + S.D., $n = 6$). ●: Control (diltiazem 15 mg kg^{-1}, oral); ▲ : pretreated with 5 mg kg^{-1} of naringin; □ : pretreated with 15 mg kg^{-1} of naringin; ◯: i.v. injection of diltiazem (5 mg kg^{-1}).

group. The effect of naringin enhancing the oral exposure of diltiazem was not dose proportional over the dose range of 5–15 mg kg^{-1}.

The pharmacokinetic profiles of desacetyldiltiazem were also evaluated in the presence and absence of naringin (Fig. 2). As summarized in Table 2, the oral exposure of desacetyldiltiazem increased significantly ($p < 0.05$) in the presence of naringin, while the metabolite–parent ratio (M.R.) in the rats decreased by approximately 30% compared to the control group, implying that the presence of naringin could be effective to inhibit the metabolism of diltiazem. As shown in Fig. 1, while the slopes of the plasma concentration–time curves are similar in all tested cases following an oral or intravenous administration of diltiazem, the concomitant use of naringin increased the C_{max} and AUC of diltiazem with decreasing the metabolite–parent ratio. Those results suggest that naringin could reduce the presystemic metabolism during the intestinal absorption. Lee et al. (1991) reported that the extraction ratios of diltiazem in small intestine and liver after an oral administration to rats were about 85 and 63%, respectively, suggesting that diltiazem is highly extracted in the small intestine as well

Table 1
Mean pharmacokinetic parameters of diltiazem following an intravenous (5 mg/kg) or oral (15 mg/kg) administration of diltiazem to rats in the presence and absence of naringin (mean ± S.D., $n = 6$)

Parameters	Diltiazem (control)	Diltiazem + naringin		Diltiazem (i.v.)
		5 mg kg^{-1}	15 mg kg^{-1}	
T_{max} (h)	0.25	0.25	0.25	–
C_{max} (ng mL^{-1})	173 ± 41.5	362 ± 65.6*	375 ± 61.1*	–
AUC (ng h mL^{-1})	358 ± 56.9	645 ± 82.3*	682 ± 54.8*	1960 ± 445
K_a (h^{-1})	2.9 ± 0.9	5.6 ± 1.0*	5.9 ± 1.0*	–
$T_{1/2}$ (h)	13 ± 2.9	13 ± 2.8	13 ± 3.4	6.0 ± 1.6
A.B. (%)	6.09 ± 1.10	12.6 ± 1.61*	13.4 ± 1.13*	–
R.B. (%)	100	207	220	–

A.B.: absolute bioavailability, R.B.: relative bioavailability compared to the control group.
* $p < 0.05$, significant difference compared to the control (given diltiazem alone orally).

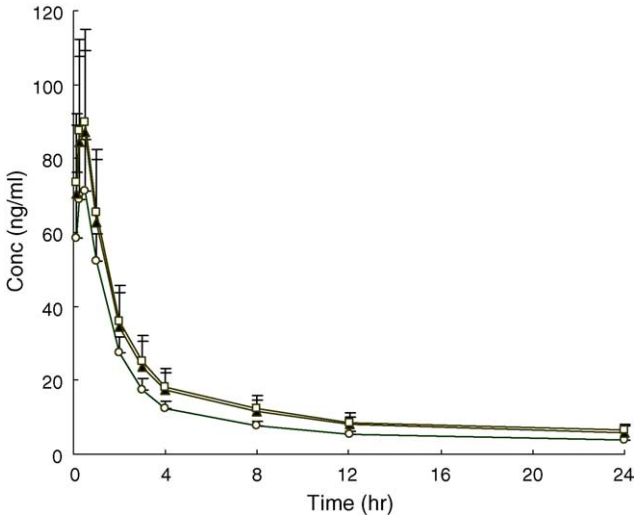

Fig. 2. Mean plasma concentration–time profiles of desacetyldiltiazem after an oral administration of diltiazem (15 mg kg^{-1}) to rats in the presence and absence of naringin (mean + S.D., $n = 6$). ●: Control (diltiazem 15 mg kg^{-1}, oral); ▲ : pretreated with 5 mg kg^{-1} of naringin; □ : pretreated with 15 mg kg^{-1} of naringin.

as in the liver. Therefore, the decrease of intestinal extraction by the concomitant use of naringin resulted in the enhanced oral bioavailability of diltiazem. The effect of naringin on the intestinal metabolism is further supported by the previous report that naringin was effective to alter the pharmacokinetics of quinine after oral administration of quinine but not after an intravenous administration in rats (Zhang et al., 2000).

Taken all together, results from the present study are consistent with the previous studies reported by Christensen et al. (2002). In their studies, the concomitant intake of grapefruit juice caused a statistically significant increase in the systemic exposure of diltiazem in healthy subjects. Also, Shin et al. (2005) have reported that naringin could be effective to enhance the oral exposure of paclitaxel prodrug by inhibition of P-gp and metabolic enzymes. In contrast, Sigusch et al. (1994) has reported that grapefruit juice did not alter AUC and C_{max} of diltiazem. In addition, in their studies, the metabolite–parent ratios of diltiazem were not affected by grapefruit juice intake, indicating that the metabolic pathway of diltiazem was not inhibited by the concomitant intake of grapefruit juice. Although the explanation on this discrepancy is not clear yet, difference in the concentrations of active constituents such as naringin, may explain some contradictory results of

Table 2
Mean pharmacokinetic parameters of desacetyldiltiazem following an oral administration of diltiazem (15 mg kg^{-1}) to rats in the presence and absence of naringin (mean ± S.D., $n = 6$)

Parameters	Diltiazem (control)	Diltiazem + naringin	
		5 mg kg^{-1}	15 mg kg^{-1}
T_{max} (h)	0.5	0.5	0.5
C_{max} (ng mL^{-1})	71.3 ± 14.5	87.7 ± 18.6	90.1 ± 20.8
AUC (ng h mL^{-1})	308 ± 60.4	438 ± 107*	469 ± 90.2*
$T_{1/2}$ (h)	13 ± 1.8	14 ± 0.9	15 ± 3.1
M.R.	0.93 ± 0.15	0.68 ± 0.13*	0.69 ± 0.14*

M.R. (metabolite–parent ratio): ($AUC_{desacetyldiltiazem}/AUC_{diltiazem}$) × ($M.W._{diltiazem}/M.W._{desacetyldiltiazem}$).
* $p < 0.05$, significant difference compared to the control (given diltiazem alone orally).

drug interaction studies with grapefruit juice, since the contents of grapefruit constituents in commercial juice and fresh grapefruit varied from brand to brand and also from lot to lot (Ho et al., 2000). For example, the naringin content, after hand-squeeze, ranged from 115 to 384 mg/L and the different lots showed variation in content (1.5-, 2.3-, and 4.7-fold for naringin, naringenin and bergapten, respectively) (Ho et al., 2000).

Although potential adverse effects, this interaction may provide a therapeutic benefit whereby it enhances oral exposure and lowers the dose administered. Since the present study raised the awareness about the potential drug interactions by concomitant use of naringin, a naturally occurring flavonoid, with diltiazem, the clinical significance of this finding need to be further evaluated in the clinical studies.

4. Conclusion

Pretreatment of naringin over the dose range of 5–15 mg/kg significantly enhanced the oral exposure of diltiazem. Therefore, concomitant use of naringin or naringin-containing dietary supplements with diltiazem may require close monitoring for potential drug interactions.

Acknowledgement

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