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Role of kaempferol to increase bioavailability and pharmacokinetics of nifedipine in rats

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[ABSTRACT] Herein, the purpose of this study is to evaluate the effects of kaempferol on bioavailability and pharmacokinetics of nifedipine and its metabolite dehydronifedipine in rats. The experimental design is based on with or without kaempferol in the oral and intravenous administration of nifedipine in rats. Moreover, the pharmacokinetic parameters including nifedipine and dehydronifedipine were evaluated in rats. The *in vitro* studies of kaempferol were investigated on P-glycoprotein (P-gp) and cytochrome P450 (CYP) 3A4 activity. Kaempferol reduced a 50% inhibitory concentration (IC_{50}) of 8.6 μmol·L^{-1} on CYP3A4 enzyme activity. Moreover, kaempferol clearly improved the cell internalization of rhodamine-123 in MCF-7/ADR cells overexpressing P-gp. Depending on increased concentrations of kaempferol, the areas under the plasma concentration–time curve ($AUC_{0-\infty}$) and the peak concentration (C_{max}) of nifedipine were increased after oral and intravenous administration. Moreover, the absolute bioavailability (AB) and relative bioavailability (RB) of nifedipine in the presence of kaempferol was significantly higher than those of the control group after oral and intravenous administration. Improvement of bioavailability of nifedipine by kaempferol may be mainly because of the inhibition of the P-gp-mediated efflux transporter in the small intestine and CYP3A4-mediated metabolism in the small intestine or liver, or both.

[KEY WORDS] Nifedipine; Kaempferol; CYP3A4; P-gp; Pharmacokinetics; Bioavailability

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Introduction

Recently, the interest in fruits or vegetables as an alternative medicine has been growing [1]. Thus, there are increasing studies to identify plant-based natural compounds that regulate P-gp and metabolic enzymes. Nevertheless, the pharmacokinetic interactions between fruit or vegetable constituents and drugs were not well studied. Therefore, preclinical and clinical studies are required for the interaction between the fruit or vegetable ingredients and the drugs.

Kaempferol (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a flavonoid, that abundantly found in onions (832 mg·kg^{-1}), carrots (140 mg·kg^{-1}), and black (118 mg·kg^{-1}) [2]. In the small intestine, P-gp is located on the apical membrane of the cell with CYP3A4 [3-4]. P-gp and CYP3A4 can synergize to limit oral absorption and first-pass metabolism [4]. Kaempferol was reported as a substrate for P-gp [5].

Nifedipine is extensively used for the clinical treatment of vascular diseases included hypertension, coronary artery spasm, and angina pectoris in classification of calcium channel–blocker [6]. Nifedipine physically blocks channels to inhibit extracellular calcium influx through myocardial and vascular membranes, reduce intracellular calcium levels, inhibit smooth muscle cells contraction, and dilate the coronary and systemic arteries [7].

Nifedipine is mainly metabolized by cytochrome P450 (CYP) 3A4, a major pyridine metabolite, dehydronifedipine [8]. CYP enzymes play a main role in the phase I metabolism of the drug and are responsible for the oxidation metabolism of many xenobiotics [9]. CYP3A3 is located in the liver and metabolizes more than 50% of the drugs including nifedipine and erythromycin [10-11]. Nifedipine is known to be a substrate for CYP3A4 in humans [10].

P-gp is a member of the adenosine triphosphate (ATP) dependent transmembrane efflux pump. P-gp has been observed in the gastrointestinal epithelium, canalicular membrane of the liver and kidney in normal tissues [12-13].

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P-gp plays a pharmacokinetic role because it belongs to various tissues and has substrate [14-15]. The substrates and/or inhibitors of CYP3A4 and P-gp were known to overlap each other overlap with each other [16]. In previous studies, kaempferol exhibited a remarkable inhibition of the P-gp-mediated efflux of ritonavir, thereby increasing its cellular uptake [17]. Thus, kaempferol would be expected to alter the pharmacokinetics of nifedipine by inhibiting P-gp and CYP3A4 in rats.

Therefore, the aim of this study was to evaluate the CYP3A4 and P-gp activity on the effects of kaempferol. In addition, we evaluated the bioavailability and pharmacokinetics of orally and intravenously administered nifedipine in rats with or without kaempferol

Material and Methods

Materials

Nifedipine, dehydronifedipine, kaempferol and amlodipine [internal standard for the high-performance liquid chromatography (HPLC) analysis of nifedipine] were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Methanol, isooctane, methyl-*tert*-butyl ether (MTBE), analytical grade acetic acid and triethylamine (TEA) were obtained from Merck Co. (Darmstadt, Germany). Rhodamine was obtained from Calbiochem (San Diego, CA, USA), and the CYP inhibition assay kit was obtained from Gentest Co. (Woburn, MA, USA). Other chemicals used were of reagent or HPLC grade. The apparatus used in this study included an HPLC system equipped with a Waters 1515 isocratic HPLC pump, a Waters 717 plus autosampler, a Waters™ 2487 scanning UV detector (Waters Co., Milford, MA, USA), an HPLC column temperature controller (Phenomenex Inc., CA, USA), a Bransonic® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortexmixer (Scientific Industries Co., NY, USA), and a high-speed microcentrifuge (Hitachi Co., Tokyo, Japan).

Animal

All animal study protocols were approved by the Animal Care Committee of Chosun University (Gwangju, Republic of Korea). The experiment was carried out in accordance with the guidelines of the National Institutes of Health (NIH publication No. 8623, revised 1985). Male Sprague–Dawley rats (250–280 g) were purchased from the Dae Han Laboratory Animal Research Co. (Eumsung, Republic of Korea). Animals were allowed free access to a normal standard chow diet (No. 322-7-1; Superfeed Co., Wonju, Republic of Korea) and tap water. The animals were housed, four or five per cage, in laminar flow cages maintained at a temperature of 22 ± 2 °C, 50%–60% relative humidity, and under a 12 h light-dark cycle. The rats were adapted for at least a week under these conditions.

The rats were fasted for at least 24 h prior to the experiments. The left femoral artery (for blood sampling) and left femoral vein (for drug administration in the intravenous study) were cannulated using an SP45 polyethylene tube (inner diameter, 0.58 mm, outer diameter, 0.96 mm; Natsume Seisakusho Company, Tokyo, Japan) while each rat was under CO_2 gas anesthesia.

Intravenous and oral administration of nifedipine

The rats were divided into six groups (n = 6, in each) as follows: oral groups administered [10 mg·kg^{-1} of nifedipine dissolved in distilled water emulsified with 10 μL tween 80 (1.0 mL·kg^{-1})] without (control) or with 0.4, 2.0 or 10 mg·kg^{-1} of kaempferol (emulsified with 10 μL tween 80; total oral volume of 1.0 mL·kg^{-1}), and intravenous groups administered (2.5 mg·kg^{-1} of nifedipine in 0.9% NaCl-injectable solution; emulsified with 10 μL tween 80, 1.0 mL·kg^{-1}) without (control) or with 0.4, 2.0 or 10 mg·kg^{-1} of kaempferol (emulsified with 10 μL tween 80). A feeding tube was used to administer nifedipine and kaempferol intragastrically. Kaempferol was administered 30 min prior to oral administration of nifedipine. Blood samples (0.5 mL) were collected in heparinized tubes *via* the femoral artery at 0.017 (end of drug infusion), 0.1, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h for the intravenous study, and 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, and 24 h for the oral study. Whole blood (approximately 1.2 mL) collected from untreated rats was infused via the femoral artery at 0.75, 4, and 8 h to replace blood loss from blood sampling. The blood samples were centrifuged (13 000 r·min^{-1}, 3 min), and 200-μL aliquots of plasma samples were stored in the deep freezer at –40 °C until the HPLC analysis.

HPLC analysis

The plasma concentrations of nifedipine in rats were determined using an HPLC assay with some modified the previous report [18]. Briefly, 50 μL of amlodipine (3 μg·mL^{-1}), as the internal standard and 50 μL of 1.0 mol·L^{-1} sodium hydroxide were added to 0.2 mL of the plasma samples and mixed for 3 s and 5 mL of MTBE−isooctane (75 : 25, *V/V*) was added. The resultant mixture was vortex-mixed for 1 min and centrifuged at 3000 r·min^{-1} for 5 min. The organic layer (0.8 mL) was transferred into a clean test tube and evaporated under a gentle stream of nitrogen gas at room condition. The dried extracts were reconstituted with 200 μL of the mobile phase vortex-mixed for 1 min. The aliquots of 160 μL were transferred to a clean HPLC vials. The supernatant (70 μL) was injected into the HPLC system. The UV detector wavelength was set to 350 nm, and a Nova-pack C_8 column (100 mm × 8 mm I.D., 4 μm; Waters Co., Milford, MA, USA) was used at room temperature. A mixture of methanol and water (62 : 38, *V/V*, pH 4.5, adjusted with acetic acid, 320 μL TEA/1000 mL of mixture was added) was used as the mobile phase with a flow rate of 1.0 mL·min^{-1}. The retention times were as follows: 16.8 min for internal standard, 8.2 min for nifedipine, and 6.5 min for dehydronifedipine. The detection limits of nifedipine and dehydronifedipine in rat plasma were all 5 ng·mL^{-1}. The coefficients of variation for nifedipine and dehydronifedipine were all below 5.0%.

CYP3A4 inhibition assay

This experiment was performed to study the inhibitory effect of kaempferol on CYP3A4 activity, as nifedipine and

kaempferol are both substrates of CYP3A4. The inhibition assay for the human CYP3A4 enzyme activity was performed using a CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously [19]. Briefly, human CYP enzyme was obtained from baculovirus-infected insect cells. The CYP substrate (7-BFC for CYP3A4) was incubated with or without the test compounds in the enzyme/substrate buffer with 1 pmol of P450 enzyme and an NADPH-generating system (1.3 mmol·L^{-1} NADP, 3.54 mmol·L^{-1} glucose 6-phosphate, 0.4 U·mL^{-1} glucose 6-phosphate dehydrogenase and 3.3 mmol·L^{-1} $MgCl_2$) in potassium phosphate buffer (pH 7.4). After 45 min, reactions were terminated by adding the stop solution. Metabolite concentrations of samples were measured with a spectrofluorometer (Molecular Device, Sunnyvale, CA, USA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. The positive control (1 μmol·L^{-1} ketoconazole for CYP3A4) was run on the same plate and showed 99% inhibition. All experiments were done in duplicate, and the results were expressed as percentage inhibition.

Rhodamine-123 retention assay

This study was conducted to investigate the effect of kaempferol on P-gp since kaempferol is a substrate for P-gp. The procedures used for the Rhodamine-123 retention assay were similar to previously reported methods [20]. The MCF-7/ADR cells were seeded 3 × 10^5 in 24-well plates. At 80% confluence, the cells were incubated in fetal bovine serum (FBS)-free Dulbecco's modified Eagle's medium (DMEM) for 18 h. The culture medium was changed to Hanks' balanced salt solution (HBSS) and the cells were incubated at 37 °C for 30 min. Following incubation of the cells with 20 μmol·L^{-1} rhodamine-123 in the presence or absence of kaempferol (0, 50 and 100 μmol·L^{-1}) and verapamil (positive control, 100 μmol·L^{-1}) for 90 min, the medium was completely removed. The cells were then washed three times with ice-cold phosphate buffer (pH 7.0) and lysed in EBC lysis buffer. Rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and were presented as a ratio to the control.

Pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using the Thermo Kinetica Software Version 5.0 (Thermo Fisher Scientific Inc., Miami, OK, USA). The parameter values were obtained by fitting the data to the pharmacokinetic model using the simplex algorithm. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule-extrapolation method [21]. The peak concentration (C_{max}) of nifedipine in plasma and the time to reach C_{max} (T_{max}) were obtained by visual inspection of the data from the concentration–time curve. The terminal half-life ($t_{1/2}$) was calculated by 0.693/K_{el}. The total body clearance (CL/F) was calculated by dose/AUC. The absolute bioavailability (AB) was calculated by $AUC_{oral}/AUC_{i.v.} \times dose_{i.v.}/dose_{oral}$, and the relative bioavailability (RB) of nifedipine were calculated by $AUC_{nifedipine\ with\ kaempferol}/AUC_{control}$. The metabolite–parent AUC ratio (MR) was calculated by $AUC_{dehydronifedipine}/AUC_{nifedipine}$.

Statistical analysis

The data are presented as the mean ± standard deviation (SD). The pharmacokinetic parameters were compared using a one-way analysis of variance (ANOVA), followed by a posteriori testing with the use of the Dunnett correction. A P value of < 0.05 was considered statistically significant.

Results

Effect of kaempferol on CYP3A4 activity

The inhibitory effect of kaempferol on CYP3A4 activity was shown in Fig. 1. The positive control as ketoconazole inhibited CYP3A4 activity with IC_{50} value of 0.14 μmol·L^{-1}. Kaempferol inhibited CYP3A4 activity depended on concentration manner with an IC_{50} value of 8.6 μmol·L^{-1}. Although the IC50 of kaempferol was lower than that of ketoconazole, the inhibitory effect of CYP3A4 in kaempferol was confirmed.

Effect of kaempferol on the P-gp activity

The accumulation of rhodamine-123, a P-gp substrate, was increased in MCF-7/ADR cells overexpressing P-gp compared to the accumulation observed in MCF-7 cells lacking P-gp, as shown in Fig. 2. The concurrent use of kaempferol (P < 0.05 for 100 μmol·L^{-1}) enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner. This result suggests that kaempferol significantly inhibited P-gp activity.

Effects of kaempferol on the pharmacokinetics of oral nifedipine

The mean plasma concentration-time profiles of nifedipine in the presence and absence of kaempferol (0.4, 2.0 and 10 mg·kg^{-1}) are shown in Fig 3. The pharmacokinetic parameters of nifedipine are summarized in Table 1. Kaempferol significantly (2.0 mg·kg^{-1}, P < 0.05; 10.0 mg·kg^{-1}, P < 0.01) increased the $AUC_{0-\infty}$ of nifedipine and the C_{max} of nifedipine by 38.1%–55.7% and 19.6%–52.4% respectively. The CL/F was significantly decreased (26.0%−35.6%) by kaempferol at doses of 2.0 mg·kg^{-1} (P < 0.05) and 10.0 mg·kg^{-1} (P < 0.01). The AB values of nifedipine in the presence of kaempferol (2.0, or 10 mg·kg^{-1}) were significantly higher (34.8%–55.7%, P < 0.05; P < 0.01) than those in the control group. Kaempferol increased the RB of nifedipine 1.38–1.56 fold. In summary, The $AUC_{0-\infty}$, AB values, and RB values were significantly increased depended on kaempferol concentration manners compared to those of the control group.

Effect of kaempferol on the pharmacokinetics of dehydronifedipine

The plasma concentration-time profiles of dehydronifedipine are shown in Fig 4. The pharmacokinetic parameters of dehydronifedipine are summarized in Table 2. Kaempferol increased the $AUC_{0-\infty}$ of dehydronifedipine, but this was not significant. The MR ratios were significantly

($P < 0.05$) decreased by kaempferol at doses of 2 and 10 mg·kg^{-1} by 19.5% and 25.0%, respectively. This result suggests that the formation of dehydronifedipine was considerably altered by kaempferol. The increase in the bioavailability of nifedipine may be mainly because of the inhibition of CYP3A4 activity by kaempferol in the small intestine or liver or both.

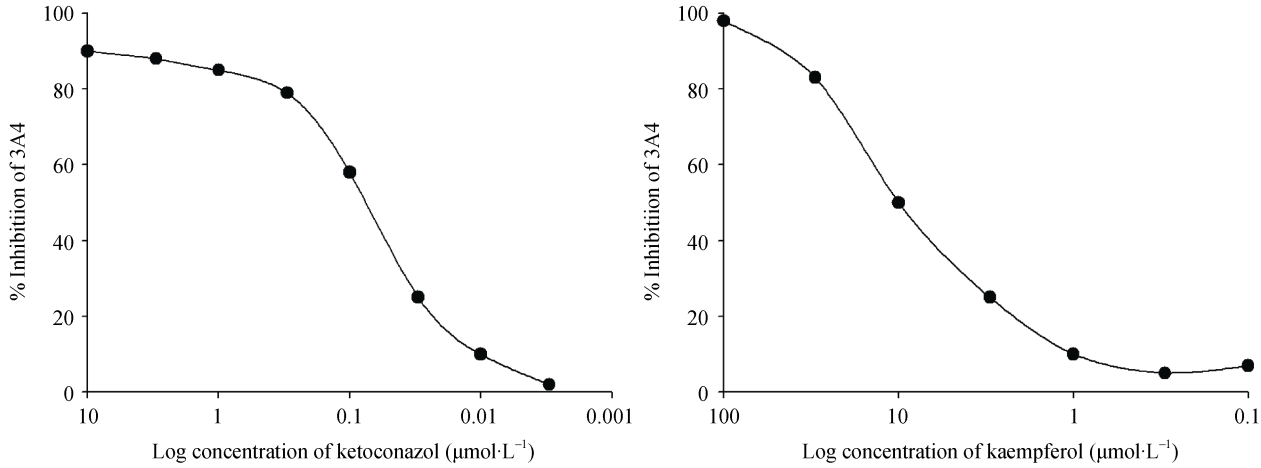

Fig. 1 Inhibitory effect of ketoconazole and kaempferol on CYP3A4 activity. The results were expressed as the percent of inhibition

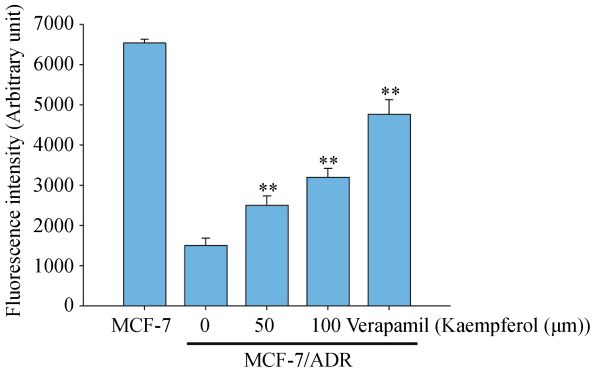

Fig. 2 Effects of kaempferol on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data are represented as the mean ± SD (n = 6). $^{*}P < 0.05$ *vs* positive control (verapamil)

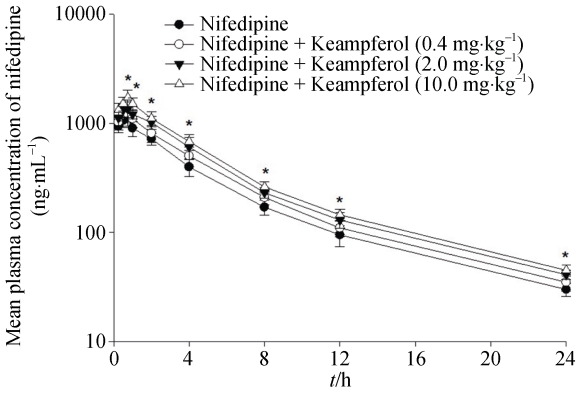

Fig. 3 Mean plasma concentration-time profiles of nifedipine after oral administration of nifedipine (10.0 mg·kg^{-1}) in rats in the presence and absence of kaempferol (0.4, 2.0 or 10.0 mg·kg^{-1}) (mean ± SD, n = 6). (●) nifedipine alone, (○) with 0.4 mg·kg^{-1} kaempferol, (▲) with 2.0 mg·kg^{-1} kaempferol, (△) with 10.0 mg·kg^{-1} kaempferol. $^{*}P < 0.05$ *vs* nifedipine

Effects of kaempferol on the pharmacokinetics of intravenous nifedipine

The mean arterial plasma concentration-time profiles of nifedipine following its intravenous administration of nifedipine (2.5 mg·kg^{-1}) in rats in the presence or absence of kaempferol (0.4, 2.0 and 10.0 mg·kg^{-1}) are shown in Fig. 5, and the corresponding pharmacokinetic parameters are shown in Table 3. Kaempferol (2.0 and 10 mg·kg^{-1}) significantly ($P < 0.05$) increased the $AUC_{0-\infty}$ of nifedipine by 23.0%–30.1%. The CL_t was significantly decreased (19.3%–25.6%, $P < 0.05$) by kaempferol at doses of 2.0 and 10 mg·kg^{-1}. The $t_{1/2}$ of nifedipine was also increased, but this increase was not statistically significant.

Discussion

In recent years, dependence on pharmaceuticals has been gradually decreasing, and patients were looking for alternative medicines with lower drug efficacy but fewer side effects and easier to purchase. With the growing great interest in fruit or vegetable components as alternative medicine, effort is currently being put into identifying plant-based natural compounds that modulate P-gp and metabolic enzymes. However, there is very little information on the pharmacokinetic interactions between fruit or vegetable components and drugs. More preclinical and clinical investigations of the possible interactions between fruit or vegetable constituents and drugs are needed. Such studies would contribute to preventing potential adverse reactions in patients. Alternatively, this information may also be useful because the identified interactions may prove to possess potentially beneficial therapeutic applications. Therefore, the present study evaluated the effect of kaempferol (a main component of onion) on the bioavailability and pharmacokinetics of nifedipine in rats. We investigated whether the potential interactions between our hypothe-

sized kaempferol and nifedipine could be mediated through dual inhibition of CYP3A4 and P-gp. Furthermore, since kaempferol or onion juice is readily available over-the-counter, there is a possibility that kaempferol or onion juice as a health supplement may be taken along with prescribing nifedipine for the treatment of cardiovascular disease.

Table 1 Mean pharmacokinetic parameters of nifedipine after oral administration of nifedipine (10.0 mg·kg^{-1}) in rats in the presence or absence of kaempferol (mean ± SD, *n* = 6)

Parameters	Nifedipine (control)	Nifedipine + kaempferol (mg·kg^{-1})		
		0.4	2.0	10.0
$AUC_{0-\infty}$ (ng·h·mL^{-1})	5930 ± 1067	6926 ± 1246	8187 ± 1364*	9234 ± 1569**
C_{max} (ng·mL^{-1})	1130 ± 192	1222 ± 219	1352 ± 229*	1722 ± 297**
T_{max} (h)	0.71 ± 0.19	0.71 ± 0.19	0.69 ± 0.14	0.69 ± 0.14
CL/F (mL·h^{-1}·kg^{-1})	28.1 ± 5.1	24.1 ± 4.3	20.8 ± 3.5*	18.1 ± 3.1**
$t_{1/2}$ (h)	9.5 ± 1.8	9.7 ± 2.0	9.8 ± 2.0	10.1 ± 2.2
AB (%)	15.8 ± 2.7	18.4 ± 3.3	21.3 ± 3.6*	24.6 ± 4.2**
RB (%)	100	117	138	156

$^{*}P < 0.05$, $^{**}P < 0.01$ *vs* the control group given nifedipine alone; $AUC_{0-\infty}$, area under the plasma concentration-time curve; C_{max}, peak concentration; T_{max}, time to reach C_{max}; AB, Absolute bioavailability; RB, relative bioavailability; $t_{1/2}$, terminal half-life; *CL/F*, total body clearance

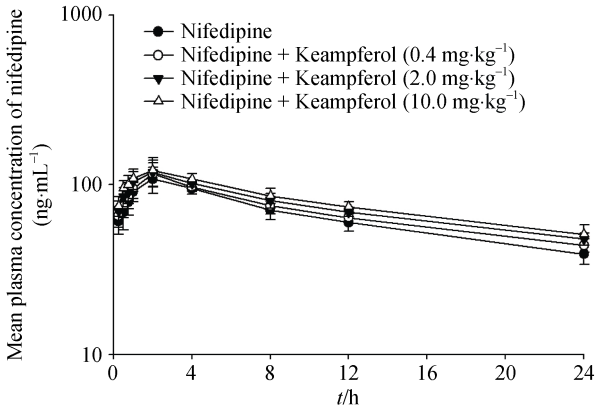

Fig. 4 Mean plasma concentration-time profiles of dehydronifedipine after oral administration of nifedipine (10.0 mg·kg^{-1}) in rats in the presence and absence of kaempferol (0.4, 2.0 or 10.0 mg·kg^{-1}) (mean ± SD, *n* = 6). (●) nifedipine alone, (○) with 0.4 mg·kg^{-1} kaempferol, (▲) with 2.0 mg·kg^{-1} kaempferol, (△) with 10.0 mg·kg^{-1} kaempferol

In Fig. 1, kaempferol (IC_{50} value of 8.6 μmol·L^{-1}) has a lower inhibitory effect on CYP3A4 than ketoconazole (IC_{50} value of 0.14 μmol·L^{-1}). When inhibitory effect on CYP3A4 activity (< 15 μmol·L^{-1} in IC_{50}), the bioavailability (*AUC*) of CYP3A4 substrates was increased by only CYP3A4 inhibitors [22-24]. Our results were similar to those of a previous paper [25]. Moreover, the pharmacokinetic parameters of nifedipine were evaluated in rats in the absence and presence of kaempferol. Human CYP2C9 and 3A4 and rat CYP2C11 and 3A4 have 77% and 73% protein homology, respectively [22, 24, 26]. In particular, CYP3A4 expressed in rats corresponds to the function of CYP3A4 in humans [22-24, 27-28].

In Fig. 2, the concentration of kaempferol (100 μmol·L^{-1}) was significantly ($P < 0.05$) inhibited P-gp activity. Our results for P-gp inhibition were similar to that of a previous paper [29]. In general, P-gp affects the absorption of drugs by efflux transport. The inhibition of P-gp activity was performed generally to compare with verapamil 100 μmol·L^{-1} standard index of inhibition [23-24]. Therefore, concomitant administration of kaempferol may affect the bioavailability and pharmacokinetics of orally administered nifedipine. Nifedipine for oral administration was a substrate for both CYP3A4-mediated metabolism and P-gp-mediated efflux in the intestine and liver.

Table 2 Mean pharmacokinetic parameters of dehydronifedipine after oral administration of nifedipine (10.0 mg·kg^{-1}) in rats in the presence or absence of kaempferol (mean ± SD, *n* = 6)

Parameters	Nifedipine (control)	Nifedipine + kaempferol (mg·kg^{-1})		
		0.4	2.0	10.0
$AUC_{0-\infty}$ (ng·h·mL^{-1})	2156 ± 385	2371 ± 399	2431 ± 465	2531 ± 498
C_{max} (ng·mL^{-1})	108 ± 19	116 ± 19	118 ± 21	120 ± 23
T_{max} (h)	2.00 ± 1.09	2.00 ± 1.09	2.17 ± 0.98	2.17 ± 0.98
$t_{1/2}$ (h)	14.9 ± 2.5	15.7 ± 2.5	16.8 ± 3.0	17.0 ± 3.2
RB (%)	100	110	113	117
MR (%)	0.36 ± 0.06	0.34 ± 0.06	0.29 ± 0.04*	0.27 ± 0.04*

$^{*}P < 0.05$ *vs* the control group given nifedipine alone; $AUC_{0-\infty}$, area under the plasma concentration-time curve; C_{max}, peak concentration; T_{max}, time to reach C_{max}; RB, relative bioavailability; $t_{1/2}$, terminal half-life; MR, metabolite-parent drug *AUC* ratio

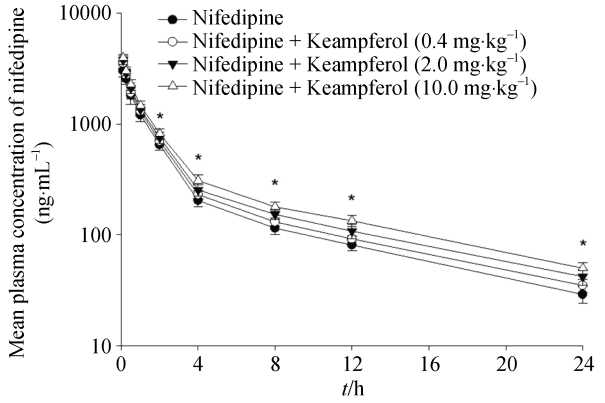

Fig. 5 Mean plasma concentration-time profiles of nifedipine after intravenous administration of nifedipine (2.5 mg·kg^{-1}) in rats in the presence and absence of kaempferol (0.4, 2.0 or 10.0 mg·kg^{-1}) (mean ± SD, n = 6). (●) nifedipine alone, (○) with 0.4 mg·kg^{-1} kaempferol, (▲) with 2.0 mg·kg^{-1} kaempferol, (△) with 10.0 mg·kg^{-1} kaempferol. $^{*}P < 0.05$ *vs* nifedipine

In Table 1, kaempferol significantly increased $AUC_{0-\infty}$ and C_{max} of nifedipine. Depending on increased kaempferol, $AUC_{0-\infty}$ and C_{max} of nifedipine were increased to 56% and 52%, respectively. The AB of nifedipine in the presence of kaempferol was significantly higher than that of control group. However, the CL/F of nifedipine was significantly decreased by kaempferol. In previous studies, grapefluit, oral diallyl trisulfide (major organosulfur compounds derived from garlic), resveratrol and quercetin significantly increased the bioavailability of losartan, nifedipine, nicardipine and tamoxifen by inhibiting CYP3A4 in rats [22, 27-28, 30]. The results of our study are almost similar to those of the above paper.

Moreover, the absolute bioavailability (AB) of nifedipine was reported to be 53% in human after oral administration [31]. As a result of our study, the AB value of nifedipine was 15.8%, indicating a difference between human and rat data. Combined administration of kaempferol increased the AB value of nifedipine by 55% and could be expected to be 53% to 80% in humans.

As summarized in Table 2, kaempferol significantly decreased metabolite-parent AUC ratios (MR). Therefore, a decrease of the MR of nifedipine in present study may be mainly due to the inhibitory effect of kaempferol on the metabolic first-pass effects in the liver or small intestine. In a previous study, myricetin significantly increased AUCs of losartan and decreased the MR of losartan in rats [23]. Therefore, our results were similar to those of the previous study.

Kaempferol significantly altered the pharmacokinetic parameters of intravenously administered nifedipine in Table 3. Kaempferol significantly increased the $AUC_{0-\infty}$ of nifedipine. However, this result is not consistent with previous studies, showing that hesperidine did not increase the AUCs of intravenous verapamil in rats [22-32]. In summary, kaempferol inhibited CYP3A4 enzyme activity and enhanced the cellular accumulation of rhodamine-123 in MCF-7/ADR cells overexpressing P-gp. Moreover, the relative bioavailability (RB) of nifedipine in the presence of kaempferol was significantly higher than those of the control group after oral and intravenous administration. The metabolite of parent drug AUC ratio (MR) in the presence of kaempferol was signifycantly decreased compared to that of control group. Therefore, this study, which increased relative bioavailability of nifedipine in the presence of kaempferol and reduces metabolite of parent drug AUC ratio (MR), will be an important basis for drug-drug interactions. Furthermore, since kaempferol (a component of onions) is readily available over-the-counter in pharmacies, there is a possibility that taking onion juice as a health supplement along with prescribing nifedipine prevent or treat cardiovascular disease such as hyperlipidemia, atherosclerosis and hypertension.

Table 3 Mean pharmacokinetic parameters of nifedipine after intravenous (2.5 mg·kg^{-1}) administration in rats in the presence or absence of kaempferol (mean ± SD, n = 6)

Parameters	Nifedipine (control)	Nifedipine + kaempferol (mg·kg^{-1})		
		0.4	2.0	10.0
$AUC_{0-\infty}$ (ng·h·mL^{-1})	9426 ± 1508	10466 ± 1679	11597 ± 1818*	12259 ± 1846*
CL_t (mL·h^{-1}·kg^{-1})	8.8 ± 1.4	7.8 ± 1.3	7.1 ± 1.1*	6.6 ± 1.0*
$t_{1/2}$ (h)	9.3 ± 1.7	9.5 ± 1.8	10.3 ± 2.0	10.3 ± 1.9
RB (%)	100	111	123	130

$^{*}P < 0.05$ *vs* the control group given nifedipine alone; $AUC_{0-\infty}$, area under the plasma concentration-time curve; RB, relative bioavailability; $t_{1/2}$, terminal half-life; CL_t, total body clearance

Conclusion

Kaempferol significantly increased $AUC_{0-\infty}$ and C_{max} of nifedipine after oral and intravenous administration. The increased bioavailability of nifedipine with kaempferol may be mainly due to the inhibition of the P-gp-mediated efflux transporter in the small intestine as well as the inhibition of CYP3A4-mediated metabolism in the small intestine or liver or both. Moreover, the decrease of total body clearance of nifedipine by kaempferol may also contribute to the increased

bioavailability. In this study raised the awareness of the potential drug interactions with the concomitant use of kaempferol and nifedipine. The clinical significance of this finding needs to be further evaluated in future studies.

References

[1] Brandão DO, Guimarães GP, Santos RL, *et al.* Model analytical development for physical, chemical, and biological characterization of Momordica charantia vegetable drug [J]. *J Anal Methods Chem*, 2016, **1-15**.

[2] Miean KH, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants [J]. *J Agric Food Chem*, 2001, **49**(6): 3106-3112.

[3] Cummins CL, Jacobsen W, Benet LZ. Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4 [J]. *J Pharmacol Exp Ther*, 2002, **300**(3): 1036-1045.

[4] Watkins PB. The barrier function of CYP3A4 and P-glycoprotein in the small bowel [J]. *Adv Drug Deliv Rev*, 1997, **27**(2): 161-170.

[5] Wang Y, Cao J, Zeng S. Involvement of P-glycoprotein in regulating cellular levels of Ginkgo flavonols: quercetin, kaempferol, and isorhamnetin [J]. *J Pharm Pharmacol*, 2005, **57**(6): 751-758.

[6] Sorkin E, Clissold S, Brogden R. Nifedipine [J]. *Drugs*, 1985, **30**(3): 182-274.

[7] Blackshear JL, Orlandi C, Williams GH, *et al.* The renal response to diltiazem and nifedipine: comparison with nitroprusside [J]. *J Cardiovasc Pharmacol*, 1986, **8**(1): 37-43.

[8] Funaki T, Soons P, Guenoerich F, *et al. In vivo* oxidative cleavage of a pyridinecarboxylic acid ester metabolite of nifedipine [J]. *Biochem Pharmacol*, 1989, **38**(23): 4213-4216.

[9] He F, Bi Hc, Xie Zy, *et al.* Rapid determination of six metabolites from multiple cytochrome P450 probe substrates in human liver microsome by liquid chromatography/mass spectrometry: application to high-throughput inhibition screening of terpenoids [J]. *Rapid Commun Mass Spectrom*, 2007, **21**(5): 635-643.

[10] Rendic S, Carlo FJD. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors [J]. *Drug Metab Rev*, 1997, **29**(1-2): 413-580.

[11] Shimada T, Yamazaki H, Mimura M, *et al.* Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians [J]. *J Pharmacol Exp Ther*, 1994, **270**(1): 414-423.

[12] Sugawara I, Kataoka I, Morishita Y, *et al.* Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK 16 [J]. *Cancer Res*, 1988, **48**(7): 1926-1929.

[13] Thiebaut F, Tsuruo T, Hamada H, *et al.* Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues [J]. *Proc Nat Acad Sci*, 1987, **84**(21): 7735-7738.

[14] Wandel C, Kim RB, Kajiji S, *et al.* P-glycoprotein and cytochrome P-450 3A inhibition: dissociation of inhibitory potencies [J]. *Cancer Res*, 1999, **59**(16): 3944-3948.

[15] Relling MV. Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? [J]. *Ther Drug Monit*, 1996, **18**(4): 350-356.

[16] Wacher VJ, Wu CY, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: Implications for drug delivery and activity in cancer chemotherapy [J]. *Mol Carcinog*, 1995, **13**(3): 129-134.

[17] Patel J, Buddha B, Dey S, *et al. In vitro* interaction of the HIV protease inhibitor ritonavir with herbal constituents: changes in P-gp and CYP3A4 activity [J]. *Am J Ther*, 2004, **11**(4): 262-277.

[18] Grundy JS, Kherani R, Foster RT. Sensitive high-performance liquid chromatographic assay for nifedipine in human plasma utilizing ultraviolet detection [J]. *J Chromatogr B Biomed Appl*, 1994, **654**(1): 146-151.

[19] Crespi CL, Miller VP, Penman BW. Microtiter plate assays for inhibition of human, drug-metabolizing cytochromes P450 [J]. *Anal Biochem*, 1997, **248**(1): 188-190.

[20] Han CY, Cho KB, Choi HS, *et al.* Role of FoxO1 activation in MDR1 expression in adriamycin-resistant breast cancer cells [J]. *Carcinogenesis*, 2008, **29**(9): 1837-1844.

[21] Chiou WL. Critical evaluation of the potential error in pharmacokinetic studies of using the linear trapezoidal rule method for the calculation of the area under the plasma level-time curve [J]. *J Pharmacokinet Biopharm*, 1978, **6**(6): 539-546.

[22] Choi JS, Choi BC, Kang KW. Effect of resveratrol on the pharmacokinetics of oral and intravenous nicardipine in rats: possible role of P-glycoprotein inhibition by resveratrol [J]. *Pharmazie*, 2009, **64**(1): 49-52.

[23] Choi DH, Li C, Choi JS. Effects of myricetin, an antioxidant, on the pharmacokinetics of losartan and its active metabolite, EXP‐3174, in rats: possible role of cytochrome P450 3A4, cytochrome P450 2C9 and P‐glycoprotein inhibition by myricetin [J]. *J Pharm Pharmacol*, 2010, **62**(7): 908-914.

[24] Lee CK, Ki SH, Choi JS. Effects of oral curcumin on the pharmacokinetics of intravenous and oral etoposide in rats: possible role of intestinal CYP3A and P‐gp inhibition by curcumin [J]. *Biopharm Drug Dispos*, 2011, **32**(4): 245-251.

[25] Zhang FF, Zheng YF, Zhu HJ, *et al.* Effects of kaempferol and quercetin on cytochrome 450 activities in primarily cultured rat hepatocytes [J]. *J Zhejiang Univ Med Sci*, 2006, **35**(1): 18-22.

[26] De Montellano PRO. *Cytochrome P450: structure, mechanism, and biochemistry* [M]. Springer Science & Business Media, 2005: 122-123.

[27] Zaidenstein R, Soback S, Gips M, *et al.* Effect of grapefruit juice on the pharmacokinetics of losartan and its active metabolite E3174 in healthy volunteers [J]. *Ther Drug Monit*, 2001, **23**(4): 369-373.

[28] Shin SC, Choi JS, Li X. Enhanced bioavailability of tamoxifen after oral administration of tamoxifen with quercetin in rats [J]. *Intern J Pharm*, 2006, **313**(1): 144-149.

[29] Kitagawa S, Nabekura T, Takahashi T, *et al.* Structure–activity relationships of the inhibitory effects of flavonoids on P-glycoprotein-mediated transport in KB-C2 cells [J]. *Biol Pharm Bull*, 2005, **28**(12): 2274-2278.

[30] Wang Y, Zou MJ, Zhao N, *et al.* Effect of diallyl trisulfide on the pharmacokinetics of nifedipine in rats [J]. *J Food Sci* 2011, **76**(1).

[31] Leucuța S, Vida-Simiti L, Căprioară M, *et al.* Bioavailability of nifedipine from different oral dosage forms in healthy volunteers [J]. *Die Pharmazie*, 1989, **44**(5): 336-338.

[32] Piao YJ, Choi JS. Enhanced bioavailability of verapamil after oral administration with hesperidin in rats [J]. *Arch Pharm Res*, 2008, **31**(4): 518-522.

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