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Short Communication

Effect of hesperidin on the oral pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats

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Abstract

Objectives This study was to investigate the effect of hesperidin, an antioxidant, on the bioavailability and pharmacokinetics of diltiazem and its active major metabolite, desacetyldiltiazem, in rats.

Methods A single dose of diltiazem was administered orally (15 mg/kg) in the presence or absence of hesperidin (1, 5 or 15 mg/kg), which was administered 30 min before diltiazem.

Key findings Compared with the control group (given diltiazem alone), hesperidin (5 or 15 mg/kg) significantly altered the pharmacokinetic parameters of diltiazem, except for 1 mg/kg hesperidin. The area under the plasma concentration–time curve from time 0 h to infinity ($AUC_{0-\infty}$) was significantly (5 mg/kg, $P < 0.05$; 15 mg/kg, $P < 0.01$) increased by 48.9–65.3% and the peak plasma concentration (C_{max}) was significantly ($P < 0.05$) increased by 46.7–62.4% in the presence of hesperidin (5 or 15 mg/kg). Consequently, the absolute bioavailability (F) of diltiazem with hesperidin was significantly (5 mg/kg, $P < 0.05$; 15 mg/kg, $P < 0.01$) higher than that in the control group. Hesperidin (5 or 15 mg/kg) significantly ($P < 0.05$) increased the $AUC_{0-\infty}$ and 15 mg/kg of hesperidin significantly ($P < 0.05$) increased the C_{max} of desacetyldiltiazem. However, the metabolite–parent ratio (MR) of desacetyldiltiazem was not significantly changed in the presence of hesperidin.

Conclusions Hesperidin significantly enhanced the oral bioavailability of diltiazem in rats. It might be considered that hesperidin increased the intestinal absorption and reduced the first-pass metabolism of diltiazem in the intestine and in the liver via an inhibition of cytochrome P450 3A or P-glycoprotein.

Keywords diltiazem; hesperidin; pharmacokinetics; rats

Introduction

Diltiazem is a well-known calcium channel antagonist. It is widely used for the treatment of cardiovascular diseases such as angina, supraventricular arrhythmias and hypertension.[1–3] Diltiazem undergoes extensive presystemic metabolism, and the absolute bioavailability is approximately 40% with a large inter-individual variation.[3,4] The estimated hypotensive potency of desacetyldiltiazem, a major active metabolite of diltiazem, is about one-half that of diltiazem. But the potencies of *N*-demethyldiltiazem and *N*-demethyldesacetyl-diltiazem are about one-third the potency of diltizaem.[5,6] Diltiazem is a substrate of the cytochrome P450 (CYP) 3A4. CYP3A4 is mainly located in liver and in small intestine.[7–9] Consequently, diltiazem can be metabolized in the small intestine and liver.[10–12] It has been reported that diltiazem is highly extracted in the small intestine as well as in the liver.[13] P-glycoprotein (P-gp) can also contribute to the low bioavailability of diltiazem. It has also been reported that diltiazem is a substrate of CYP3A4 and P-gp.[14]

Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically in presystemic metabolism, limiting the absorption of drugs.[14,15]

There are a few papers on the pharmacokinetic interactions between herbal products and medicines. Some investigators have reported that the pharmacokinetic interaction between herbal constituents and drugs might be modulated by metabolizing enzymes and also drug transporters such as P-gp.[16–18] Many herbal constituents, particularly, flavonoids, have many beneficial effects such as antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic and anti-carcinogenic actions.[19,20] Hesperidin, a flavonoid and an antioxidant, is one of the main constituents of *Chinpi*, which is made of satsuma mandarin peel

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(*Citrus unshiu* Marc), and is a flavanone glycoside consisting of hesperidin aglycone and disaccharide rutinose. Hesperidin is absorbed through the gastrointestinal tract and it can be detected in urine and plasma. Some investigators found that hesperidin can regulate P-gp.[21] Furthermore, hesperidin can inhibit CYP3A4,[22] while bioflavonoids, such as hesperidin, an inhibitor of P-gp, significantly increase the permeation of vincristine across the blood–brain barrier.[23] Wacher *et al.*[14] reported that diltiazem is a substrate of both CYP3A4 and P-gp. Thus, a pharmacokinetic interaction between diltiazem and hesperidin is expected. Hesperidin and diltiazem could be prescribed for the prevention or treatment of cardiovascular disease, such as hypertension, but the effect of hesperidin on the oral bioavailability and pharmacokinetics of diltiazem has not been reported *in vivo*. Therefore, this study aimed to investigate the effect of hesperidin on the oral pharmacokinetics of diltiazem and its major active metabolite, desacetyldiltiazem, in rats.

Materials and Methods

Chemicals and apparatus

Diltiazem hydrochloride, desacetyldiltiazem, hesperidin and imipramine hydrochloride (an internal standard for the high-performance liquid chromatographic (HPLC) analysis) were purchased from Sigma-Aldrich Co. (St Louis, USA). Acetonitrile, methanol and *tert*-butylmethylether were obtained from Merck Co. (Darmstadt, Germany). Other chemicals were of reagent grade or HPLC grade.

The apparatus used in this study was a high-performance liquid chromatograph equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus autosampler and a Waters 474 scanning fluorescence detector (Waters Co., Milford, USA) and an HPLC column temperature controller (Phenomenex Inc., Torrance, USA). A Bransonic ultrasonic cleaner (Branson Ultrasonic Co., Danbury, USA), a vortex-mixer (Scientific Industries Co., New York, USA) and a high-speed micro-centrifuge (Hitachi Co., Tokyo, Japan) were also used.

Animal experiments

Male Sprague–Dawley rats, 7–8 weeks old, 270–300 g, were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Republic of Korea) and given free access to commercial rat chow (No. 322-7-1; Superfeed Co., Gangwon, Republic of Korea) and tap water. The rats were housed, four or five per cage, in laminar flow cages maintained at 22 ± 2°C and 50–60% relative humidity under a 12-h light–dark cycle. The experiments began after acclimation to these conditions for at least one week and were carried out in accordance with the 'Guiding Principles in the Use of Animals in Toxicology' adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The Animal Care Committee of Chosun University (Gwangju, Republic of Korea) approved the design and the conduct of this study.

Drug administration

In a previous study by Yeung *et al.*[24] oral administration of 15 mg/kg diltiazem to rats achieved plasma levels comparable with therapeutic concentrations in humans. Therefore, in this study, rats ($n = 6$ per each treatment) were orally given 15 mg/kg of diltiazem with either (1) hesperidin (1, 5 or 15 mg/kg), or (2) no concomitant treatment (diltiazem alone). The rats were divided into four groups each for oral and intravenous diltiazem (control 15 mg/kg for oral; 5 mg/kg for intravenous), and with 1, 5 or 15 mg/kg of orally administered hesperidin. The rats were fasted for at least 24 h before beginning the experiments and had free access to tap water. Each rat was lightly anaesthetized with ether, and the femoral artery and vein were cannulated using polyethylene tubing (SP45; i.d. 0.58 mm, o.d. 0.96 mm; Natsume Seisakusho Co. Ltd., Tokyo, Japan) for blood sampling and intravenous administration. Diltiazem (dissolved in 0.9% NaCl-injectable solution) was injected at a dose of 5 mg/kg (total injection volume, 1.5 ml/kg) over 1 min via the femoral vein (control) or 30 min after administration of 1, 5 or 15 mg/kg of hesperidin (dissolved in distilled water) through a feeding tube ($n = 6$, each). Blood samples (0.5 ml) were collected into heparinized tubes via the femoral artery at 0 (as a control), 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after oral administration of diltiazem. Blood samples were immediately centrifuged for 5 min at 13 000 rev/min and 0.2-ml samples of plasma were stored at −40°C until HPLC analysis of diltiazem and desacetyldiltiazem.

HPLC assay

The plasma concentration of diltiazem was determined using the HPLC assay by Goebel & Kolle[25] with modification. Briefly, 50 μl of imipramine (2 μg/ml), as the internal standard, and 1.2 ml of *tert*-butylmethylether were added to the 0.2-ml plasma samples. The mixture was then stirred for 2 min and centrifuged at 13 000 rev/min for 5 min. One millilitre of the organic layer was transferred to a clean test tube and 0.2 ml of 0.01 M HCl was added and mixed for 2 min. Fifty microlitres 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 were: 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 and the limit assay of diltiazem and desacetyldiltiazem was 5 ng/ml. The intra- and inter-day ($n = 5$) coefficients of variation were less than 5% for diltiazem and desacetyldiltiazem.

Pharmacokinetic analysis

The plasma concentration data were analysed using a non-compartmental method on WinNonlin software version 4.1 (Pharsight Co., Mountain View, USA). The elimination rate constant (K_{el}) was calculated by the log-linear regression of diltiazem and desacetyldiltiazem concentration data during the elimination phase, and the terminal half-life (t½)

was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and time to reach the peak concentration (T_{max}) of diltiazem and desacetyldiltiazem in plasma were obtained by visual inspection of the data in the concentration–time curve. The area under the plasma concentration–time curve (AUC_{0-t}) from time zero to the time of the last measured concentration (C_{last}) was calculated using the linear trapezoidal rule. The AUC zero to infinity ($AUC_{0-\infty}$) was obtained by adding AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. The absolute bioavailability (F), relative bioavailability (RB) and the metabolite–parent ratio (MR) were calculated, respectively, as follows:

$$F = [(AUC_{oral}/AUC_{IV}) \times (Dose_{i.v.}/Dose_{oral})] \times 100 \quad (1)$$

$$RB = (AUC_{control}/AUC_{with\ hesperidin}) \times 100 \quad (2)$$

$$MR = (AUC_{desacetyldiltiazem}/AUC_{diltiazem}) \times 100 \quad (3)$$

Statistical analysis

All the means are presented with their standard deviation. The pharmacokinetic parameters were compared through one-way analysis of variance, followed by a-posteriori testing with the use of the Dunnett correction. $P < 0.05$ was considered statistically significant.

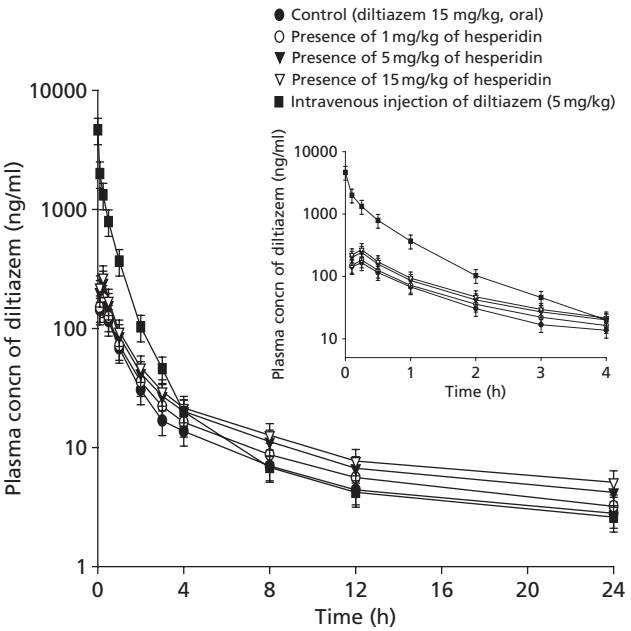

Figure 1 Plasma–concentration profile of diltiazem in rats in the presence and absence of hesperidin. Mean plasma concentration–time profile of diltiazem was determined following intravenous (5 mg/kg) or oral (15 mg/kg) administration of diltiazem to rats in the presence and absence of hesperidin. Data are presented as mean ± SD, $n = 6$.

Results

The mean plasma concentration–time profiles of diltiazem after oral administration (15 mg/kg) in the presence or absence of hesperidin (1, 5 or 15 mg/kg) were characterized in rats (Figure 1). The pharmacokinetic parameters of diltiazem are summarized in Table 1. The area under the plasma concentration–time curve from time 0 h to infinity ($AUC_{0-\infty}$) was significantly (5 mg/kg, $P < 0.05$; 15 mg/kg, $P < 0.01$) increased by 48.9–65.3%, and the peak concentration (C_{max}) was significantly ($P < 0.05$) increased by 46.7–62.4% in the presence of hesperidin (5 or 15 mg/kg). Consequently, the absolute bioavailability (F) of diltiazem was significantly (5 mg/kg, $P < 0.05$; 15 mg/kg, $P < 0.01$) higher than that in the control group, and the relative bioavailability (RB) of diltiazem was increased by 1.49–1.65 fold by hesperidin. However, there was no significant change in the time to reach the peak plasma concentration (T_{max}) or the terminal plasma half-life ($t½$) of diltiazem in the presence of hesperidin.

The pharmacokinetic profile of desacetyldiltiazem was also evaluated in the presence or absence of hesperidin (Figure 2). As summarized in Table 2, the presence of hesperidin (5 or 15 mg/kg) significantly ($P < 0.05$) increased the $AUC_{0-\infty}$ of desacetyldiltiazem by 30.1–53.8%, and the presence of hesperidin (15 mg/kg) significantly ($P < 0.05$) increased the C_{max} of desacetyldiltiazem by 19.8%. The presence of hesperidin did not significantly change the T_{max} and $t½$ of desacetyldiltiazem. Compared with the

Table 1 Pharmacokinetic parameters of diltiazem in rats in the presence or absence of hesperidin

Parameter	Diltiazem (control)	Diltiazem + hesperidin			Diltiazem (i.v.)
		1 mg/kg	5 mg/kg	15 mg/kg	
$AUC_{0-\infty}$ (ng/ml h)	352 ± 88	412 ± 103	524 ± 131*	582 ± 146**	1625 ± 408
C_{max} (ng/ml)	165 ± 42.9	183 ± 47.6	242 ± 62.9*	268 ± 69.7*	ND
T_{max} (h)	0.33 ± 0.13	0.33 ± 0.13	0.29 ± 0.10	0.29 ± 0.10	ND
t½ (h)	10.4 ± 2.7	10.5 ± 2.7	11.1 ± 2.9	11.3 ± 2.9	7.8 ± 2.2
F (%)	7.2 ± 1.7	8.5 ± 2.0	10.8 ± 2.5*	12.0 ± 2.9**	ND
RB (%)	100	117	149	165	ND

$AUC_{0-\infty}$, area under the plasma concentration–time curve from time 0 h to infinity; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}; t½, terminal plasma half-life; F, absolute bioavailability; RB, relative bioavailability; ND, not determined. Mean pharmacokinetic parameters of diltiazem were determined after intravenous (5 mg/kg) or oral (15 mg/kg) administration of diltiazem to rats in the presence and absence of hesperidin. Data are presented as mean ± SD, $n = 6$. *$P < 0.05$, **$P < 0.01$, compared with the control (diltiazem alone).

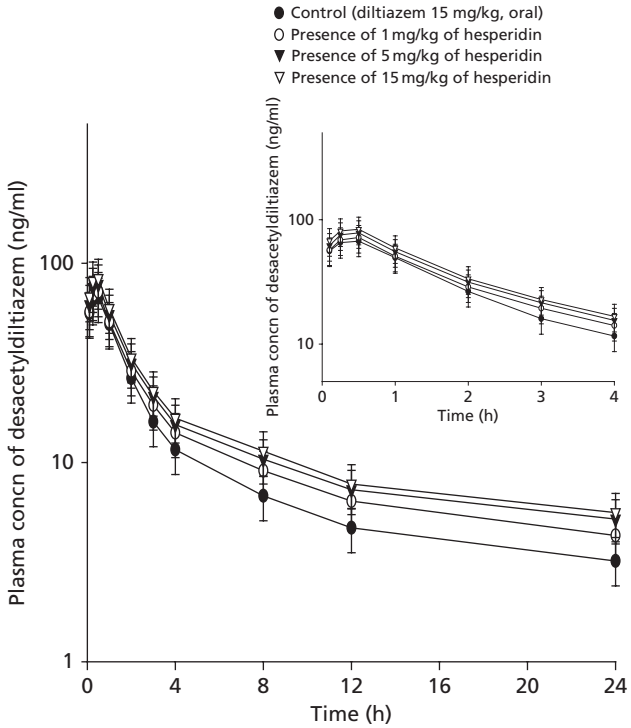

Figure 2 Plasma–concentration profile of desacetyldiltiazem in rats after oral administration of diltiazem in the presence and absence of hesperidin. Mean plasma concentration–time profile of desacetyldiltiazem was determined following oral administration of diltiazem (15 mg/kg) to rats in the presence and absence of hesperidin. Data are presented as mean ± SD, $n = 6$.

control group, the metabolite–parent AUC ratio (MR) of desacetyldiltiazem decreased in the presence of hesperidin but this change was not significant.

Discussion

With the great interest in herbal products as alternative medicines, much effort is currently being expended to identify natural plant compounds that modulate P-gp as well as metabolic enzymes; however, there is little information on the pharmacokinetic interactions between herbal products and medicines. Therefore, preclinical and clinical investigations on herbal constituent–drug interaction should be performed to prevent potential adverse reactions or to utilize those interactions for therapeutic benefit. The present study evaluated the effects of hesperidin, an antioxidant, on the oral bioavailability and pharmacokinetics of diltiazem in rats to examine potential drug interactions between diltiazem and hesperidin via dual inhibition of CYP3A4 and P-gp.

It has been reported that flavonoids can adjust the activity of P-gp and multidrug resistance associated protein 1 (MRP1), affect drug accumulation and cell viability following cytotoxic drug exposure and alter the ATPase activity of P-gp.[26,27]

Hesperidin (5 or 15 mg/kg) significantly altered the oral pharmacokinetic profile of diltiazem compared with the control. Hesperidin, an antioxidant and flavonoid, significantly enhanced the oral bioavailability of diltiazem in rats. Diltiazem is metabolized by cytochrome P450 (CYP3A) in the liver and intestinal mucosa.[7,11,12,28] Moreover, the absorption of diltiazem is inhibited by the P-gp efflux pump in the intestinal mucosa.[29,30] P-gp and CYP3A could act synergistically in intestinal first-pass metabolism. Fuhr *et al.*[22] reported that hesperidin could inhibit CYP3A, while Mitsunaga *et al.*[23] demonstrated that bioflavonoids such as hesperidin, an inhibitor of P-gp, significantly increased the permeation of vincristine across the blood–brain barrier. The pharmacokinetic parameters of diltiazem after oral administration of diltiazem were similar to those observed in previous studies.[13,24] The enhanced bioavailability of diltiazem might result from the inhibition of the P-gp efflux pump by hesperidin in the intestine; these results are consistent with those of Mitsunaga *et al.*[23] and Yusa & Tsuruo.[29] The enhanced bioavailability of diltiazem might result from the inhibition of the metabolizing enzyme CYP3A in the intestinal mucosa or in the liver by hesperidin; these results are consistent with those of Fuhr *et al.*[22] and Pichard *et al.*[7]

The above results suggest that hesperidin could effectively inhibit both the CYP3A-mediated metabolism in the small intestine or liver and the P-gp efflux transporter in the small intestine. These results were similar to those of Piao & Choi,[31] in which hesperidin enhanced the oral bioavailability of verapamil in rats.

The pharmacokinetic profile of desacetyldiltiazem was also evaluated in the presence or absence of hesperidin. The metabolite–parent ratio in the presence of hesperidin was

Table 2 Pharmacokinetic parameters of desacetyldiltiazem in rats in the presence or absence of hesperidin

Parameter	Diltiazem (control)	Diltiazem + hesperidin		
		1 mg/kg	5 mg/kg	15 mg/kg
$AUC_{0-\infty}$ (ng/ml h)	292 ± 73.0	360 ± 90.0	418 ± 105*	449 ± 112*
C_{max} (ng/ml)	67.2 ± 17.5	71.9 ± 18.7	78.4 ± 20.4	83.8 ± 21.8*
T_{max} (h)	0.46 ± 0.10	0.46 ± 0.10	0.42 ± 0.13	0.42 ± 0.13
t½ (h)	11.7 ± 3.1	12.4 ± 3.2	13.4 ± 3.5	13.5 ± 3.5
MR	0.85 ± 0.19	0.85 ± 0.20	0.80 ± 0.19	0.76 ± 0.18

$AUC_{0-\infty}$, area under the plasma concentration–time curve from time 0 h to infinity; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}; t½, terminal plasma half-life; MR, metabolite–parent ratio. Mean pharmacokinetic parameters of desacetyldiltiazem were determined following an oral administration of diltiazem (15 mg/kg) to rats in the presence and absence of hesperidin. Data are presented as mean ± SD, $n = 6$. *$P < 0.05$, compared with the control (given diltiazem alone orally).

decreased but not significantly changed compared with that in the control; these results were not similar to those of Choi & Han,[32] in which morin effectively inhibited the metabolite–parent ratio of diltiazem in rats. Hesperidin, an antioxidant and flavonoid, significantly enhanced the oral bioavailability of diltiazem in rats. It might be considered that the inhibition of CYP3A or P-gp apparently increased the intestinal absorption and reduced the first-pass metabolism of diltiazem in the small intestine and in the liver.

Conclusions

Hesperidin significantly enhanced the oral bioavailability of diltiazem in rats. It might be considered that hesperidin increased the intestinal absorption and reduced the first-pass metabolism of diltiazem in the intestine and in the liver by inhibition of CYP3A and P-gp. Therefore, concomitant use of hesperidin and diltiazem will require close monitoring for potential drug interactions.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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