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Enhanced diltiazem bioavailability after oral administration of diltiazem with quercetin to rabbits

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Abstract

The aim of this study was to investigate the effect of quercetin on the bioavailability of diltiazem after administering diltiazem (15 mg/kg) orally to rabbits either co-administered or pretreated with quercetin (2, 10, 20 mg/kg). The plasma concentrations of diltiazem in the rabbits pretreated with quercetin were increased significantly ($p < 0.05$, at 2 mg/kg; $p < 0.01$, at 10 and 20 mg/kg) compared with the control, but the plasma concentrations of diltiazem co-administered with quercetin were not significant. The areas under the plasma concentration-time curve (AUC) and the peak concentrations (C_{max}) of the diltiazem in the rabbits pretreated with quercetin were significantly higher ($p < 0.05$, at 2 mg/kg; $p < 0.01$, at 10 and 20 mg/kg) than the control. The absolute bioavailability (AB%) of diltiazem in the rabbits pretreated with quercetin was significantly ($p < 0.05$ at 2 mg/kg, $p < 0.01$ at 10 and 20 mg/kg) higher (9.10–12.81%) than the control (4.64%). AUC, AB% and C_{max} of diltiazem co-administered with quercetin were higher than the control, but these were not significant.

The bioavailibility of diltiazem in the rabbits pretreated with quercetin is increased significantly compared with the control, but not in the rabbits co-administered with quercetin. The increased bioavailability of diltiazem in the rabbits pretreated with quercetin might have been resulted result from the quercetin, which inhibits the efflux pump P-glycoprotein and the first-pass metabolizing enzyme CYP 3A4.

Keywords: Diltiazem; Quercetin; Bioavailability; Co-administration; Pretreatment

1. Introduction

Diltiazem is a calcium channel antagonist that is widely used in the treatment of angina, supraventricular arrhythmias and hypertension (Chaffman and Brogden, 1985; Pool, 1996; Weir, 1995). Diltiazem undergoes an extensive presystemic metabolism (Buckley

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et al., 1990), and the absolute bioavailability is approximately 40%, with a large inter individual variation. It was reported that in humans and dogs, *N*-demethyldiltiazem was the most abundant metabolite in plasma. In contrast, desacetyldiltiazem (DAD) and *O*-deacetyl-*N*-monodemethyl diltiazem were most predominant in the rabbits and rats, respectively (Yeung et al., 1998). CYP3A4 is the main human isoform of the *N*-demethylation of diltiazem in liver microsomes (Pichard et al., 1990). CYP 3A4 is mainly located in the liver, but it is also found in the intestine (Watkins et al., 1987; Kolars et al., 1992). Diltiazem could be metabolized in small intestine; the proximal segment is larger than the distal section (Lefebvre et al., 1996; Homsy et al., 1995a, 1995b).

The reduced bioavailability of diltiazem after administering diltiazem orally might not only be due to the metabolizing enzyme CYP 3A4 but also to the P-glycoprotein (P-gp) efflux transporter in the small intestine. Yusa and Tsuruo (1989) reported that the calcium channel blockers verapamil, nicardipine and diltiazem competitively restrain the multidrug resistance of P-gp. Saeki et al. (1993) reported that diltiazem is not only a MDR modulator but also a substrate for the efflux of P-gp. Wacher et al. (2001) also reported that diltiazem is both a CYP 3A and P-gp substrate.

P-gp is found in the secretory epithelial tissues, including the brush border of the renal proximal tubules, the canalicular membranes in the liver and the apical membranes lining the gut. In addition, it is also found in the adrenal gland, placental trophoblast and endothelial blood barrier in the brain and testes (Thiebaut et al., 1987; Cordon-Cardo et al., 1989; Sugawara et al., 1988). In the small intestine, P-gp is co-localized at the apical membrane of the cells with cytochrome P450 (CYP 3A4) (Gottesman and Pastan, 1993). P-gp and CYP3A4 might act synergistically to the presystemic drug metabolism (Gan et al., 1996; Watkins, 1996; Wacher et al., 1998; Ito et al., 1999) to make the substrate of P-gp circulate between the lumen and epithelial cells, leading to prolonged exposure to CYP 3A4, resulting in a reduced absorption of the drug.

Flavonoids represent a group of phytochemicals that are produced by various plants in high quantities (Dixon and Steele, 1999). They exhibit a wide range of beneficial biological activities including antioxidant, radical scavenging, anti-inflammatory, antiatherosclerotic, antitumoral and antiviral effects (Nijveldt et al., 2001). It has also been reported to modulate the metabolizing enzyme CYPs (Doostdar et al., 2000; Hodek et al., 2002; Dupuy et al., 2003) and inhibit the P-gp efflux pump (Dupuy et al., 2003; Bardelmeijer et al., 2000). Quercetin as an extensive class of polyphenolic flavonoid compounds that are almost ubiquitous in plants and plant food sources is the major bioflavonoid in the human diet. The estimated average daily dietary intake of quercetin by an individual in the United States is 25 mg (NTP Technical Report, 1991). It has been reported the quercetin can inhibit the P-gp pump efflux transporter (Scambia et al., 1994; Shapiro and Ling, 1997) and metabolizing enzyme, CYP 3A4 in vitro (Miniscalco et al., 1992; Guengerich and Kim, 1990). Quercetin improves the bioavailability of digoxin and moxidectin, which are substrate for P-gp and CYP 3A in the pig or lamb metabolism, respectively (Dupuy et al., 2003; Wang et al., 2004).

The bioavailability of oral diltiazem is mainly affected by CYP 3A4 and P-gp at the first-pass metabolism. When quercetin administered with diltiazem orally, it might influence the bioavailability of diltiazem. However, there has been no report about if the quercetin effluences the bioavailability of diltiazem in rabbits. The aim of this study was to examine the bioavailability of diltiazem when diltiazem was either co-administered or pretreated with quercetin.

2. Materials and methods

2.1. Materials

Diltiazem hydrochloride, imipramine hydrochloride and quercetin dihydrate (3,3′,4′,5,7-pentahydroxyflavone) was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Saline (0.9% NaCl injectable solution) was obtained from Choongwae Co. (Seoul, Korea). Acetonitrile, methanol, *tert*-butylmethylether were acquired from Merck Co. (Darmstadt, Germany). All other chemicals in this study were of reagent grade and were used without further purification. The apparatus used in this study were a high performance liquid chromatograph (LC-10AD liquid chromatograph pump, SIL-10A autoinjector, SPD-10A UV–vis detector, CBM-10A communications bus module, Shimadzu, Kyoto, Japan), a mechanical stirrer (Scientific Industries,

USA), a centrifuge (Hanil Science Industrial Co., Korea), a microcentrifuge (National Labnet, USA), a sonicator (Daihan Co., Korea) and a rotamix (SeouLin Biosience, Korea).

2.2. Animal experiments and drug administration

The male New Zealand white rabbits were purchased from Dae Han Laboratory Animal Research and Co. (Choongbuk, Korea), and were given access to a normal standard chow diet (Jae II Chow, Korea) and tap water ad libitum. Throughout the experiment, the animals were housed, two per cage, in laminar flow cages maintained at 22 ± 2 °C, 50–60% relative humidity, under a 12 h light:12 h dark cycle. The animals were kept in these facilities for at least 1 week prior to the experiment. This experiments were performed 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 in our institution (Chosun University) approved this study.

Rabbits were divided in eight groups of eight each: one of control group (diltiazem 15 mg/kg, oral), three of co-administration groups (15 mg/kg diltiazem co-administered orally with quercetin 2, 10 and 20 mg/kg, respectively), three of pretreatment groups (15 mg/kg diltiazem pretreated orally with 2, 10 and 20 mg/kg quercetin 0.5 h before, respectively), and one of i.v. group (intravenous administration of 5 mg/kg diltiazem).

Diltiazem dose (15 mg/kg) was chosen to keep plasma concentrations above the limit of detection at the time variation from 0 to 24 h in rabbits' plasma. The rabbits were fasted for at least 24 h prior to experiments and given free access to water. Each rabbit was anaesthetized by an injection of 25% urethane saline dissolution (4 ml/kg). The right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, NJ, USA) for blood sampling. Diltiazem solutions were prepared by adding diltiazem (15 mg/kg) to distilled water (10 ml) and stirring for 1 h, and then administered orally through a catheter for the control. The mixtures for co-administered group were prepared by adding diltiazem (15 mg/kg) and quercetin (2, 10, 20 mg/kg) in distilled water (10 ml) and stirred for 1 h before administration. The quercetin suspensions for pretreated groups were prepared by adding quercetin (2, 10, 20 mg/kg) to distilled water (5 ml) and stirring for 1 h, and quercetin suspensions were administered orally 30 min prior to administration of diltiazem solutions. In order to estimate the absolute bioavailability (AB%), diltiazem (5 mg/kg) was injected through the ear vein by dissolving diltiazem in the saline solution.

Blood samples (1.2 ml) were withdrawn from the femoral artery at 0, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after the oral administration of the diltiazem to each of all rabbits. The blood samples were centrifuged at 13,000 rpm for 5 min. The plasma samples (0.5 ml) were stored at −40 °C until analyzed by the HPLC.

2.3. HPLC assay

The plasma concentrations of diltiazem were determined by a HPLC assay and a modification of the method reported by Goebel and Kolle (1985). Briefly, 50 μl of imipramine (2 μg/ml), as the internal standard, and 5 ml of *tert*-butylmethylether were added to 0.5 ml of the plasma sample. It was then mixed for 20 min using a rotamix and centrifuged at 5000 rpm for 10 min. 4.5 ml of the organic layer were transferred to another capped tube, 0.3 ml of 0.01 N hydrochloride was added and the mixture was vortexed for 2 min. Fifty microlitres of the water layer were injected into the HPLC system.

The chromatographic system was composed of LC-10AD liquid chromatograph pump, SIL-10A autoinjector, SPD-10A UV–vis detector, CBM-10A communications bus module (Shimadzu, Kyoto, Japan). The detector wavelength was set to 237 nm; and the column, a μ-bondapack C_{18} (3.9 × 300 mm, 10 μm, Waters Co., Ireland) was used at room temperature. Mixtures of methanol:acetonitrile:0.04 M ammonium bromide:triethylamine (24:31:45:0.1, v/v/v, pH 7.4, adjusted with acetic acid) were used as the mobile phase at a flow rate of 1.5 ml/min. The retention times are as follows: internal standard, 10.5 min; diltiazem, 8.0 min. The calibration curve of diltiazem was linear within range 5–400 ng/ml ($r = 0.9999$). Detection limit was defined below 5 ng/ml. The within-day ($n = 5$) and day-to-day ($n = 5$) coefficients of variation were less than 5% for diltiazem and 2% for imipramine. Recovery (%) was assessed from replicate analysis ($n = 5$) for 5 days by adding 20 and 200 ng/ml of diltiazem to rabbit's plasma shown 106 ± 5.7 and 101 ± 4.9, respectively.

2.4. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed using the LAGRAN computer program (Yamaoka et al., 1981), which uses the LARGAN method to calculate the AUC of the plasma concentration (C_p) as a function of time (t). The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (T_{max}) were determined by a visual inspection of the experimental data. The elimination rate constant (K_{el}) was calculated from the slope of the line by regression analysis, and the half-life ($t_{1/2}$) of the drug was obtained by $0.693/K_{el}$. The absolute bioavailability of diltiazem after being administered orally compared to the diltiazem that is injected intravenlously was calculated as follows:

$$\text{Absolute bioavailability (AB\%)} = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{i.v.}}} \times \frac{\text{i.v. dose}}{\text{oral dose}} \times 100$$

The relative bioavailability of diltiazem administered orally was calculated as follows:

$$\text{Relative bioavailability (RB\%)} = \frac{\text{AUC}_{\text{combined}}}{\text{AUC}_{\text{control}}} \times 100$$

2.5. Statistical analysis

All the means are presented with their standard deviation (mean ± S.D.). An unpaired Student's t-test was used to determine the significant difference between the controls and the rabbits either co-administered or pretreated with quercetin. A p value < 0.05 was considered significant.

3. Results and discussion

The plasma concentrations of diltiazem after the oral administration of diltiazem (15 mg/kg), either co-administered or pretreated with various quercetin dose (2, 10 and 20 mg/kg) to rabbits are shown in Figs. 1 and 2. The bioavailability and the pharmacokinetic parameters of diltiazem after administering diltiazem, either co-administered or pretreated with quercetin are shown in Tables 1 and 2. When diltiazem (15 mg/kg) was co-administered with quercetin (2, 10 and 20 mg/kg), the plasma concentrations of diltiazem were not significant. However, it increased significantly ($p < 0.05$, at 2 mg/kg, $p < 0.01$, at 10 and 20 mg/kg) in the pretreated groups compared with the control. In the pretreated group, the AUC and C_{max} of diltiazem increased significantly in a dose-dependent manner ($p < 0.05$, at 2 mg/kg; $p < 0.01$, at 10 and 20 mg/kg) compared to the control. The $t_{1/2}$ of diltiazem pretreated with quercetin was prolonged with no significance compared to the control. The absolute bioavailability (AB%) of the diltiazem control was 4.64%, which was increased significantly ($p < 0.05$ at 2 mg/kg, $p < 0.01$ at 10 and 20 mg/kg) from 9.10 to 12.81% by pretreatment of quercetin. The relative bioavailability (RB%) of diltiazem pretreated with quercetin was 1.75–2.76 times higher than the control. By the co-administration of quercetin, the AUC and C_{max} of diltiazem were not significantly affected compared to the control.

It was reported that diltiazem is metabolized by cytochrome P-450 (CYP3A) both in the liver and small intestine (Pichard et al., 1990; Molden et al., 2002; Lefebvre et al., 1996; Homsy et al., 1995a, 1995b); and the absorption of diltiazem in the intestinal mucosa is

Table 1
Pharmacokinetic parameters of diltiazem after the oral co-administration of diltiazem (15 mg/kg) with quercetin to the rabbits

Parameters	Diltiazem control	Quercetin co-administration (mg/kg)			i.v. (5 mg/kg)
		2	10	20	
AUC (ng/ml h)	232 ± 58	287 ± 71	259 ± 66	216 ± 51	1669 ± 467
C_{max} (ng/ml)	94.2 ± 23.5	99.3 ± 24.8	95.5 ± 23.7	83.5 ± 19.4	
T_{max} (h)	0.5	0.5	0.5	0.5	
K_{el} (h^{-1})	0.061 ± 0.012	0.056 ± 0.024	0.056 ± 0.011	0.059 ± 0.018	0.124 ± 0.031
$t_{1/2}$ (h)	11.3 ± 2.8	12.3 ± 2.9	12.2 ± 2.8	11.7 ± 2.6	5.6 ± 1.4
AB%	4.64 ± 0.52	5.73 ± 0.88	5.17 ± 0.67	4.21 ± 0.42	100
RB%	100	124	112	93	

Mean ± S.D. ($n = 8$); AUC, area under the plasma concentration-time curve from 0 to 24 h. C_{max}, peak concentration; T_{max}, time to reach peak concentration; K_{el} (h^{-1}), elimination rate constant; $t_{1/2}$, terminal half-life; RB%, AUC rate compared to $AUC_{control}$; AB%, absolute bioavailability.

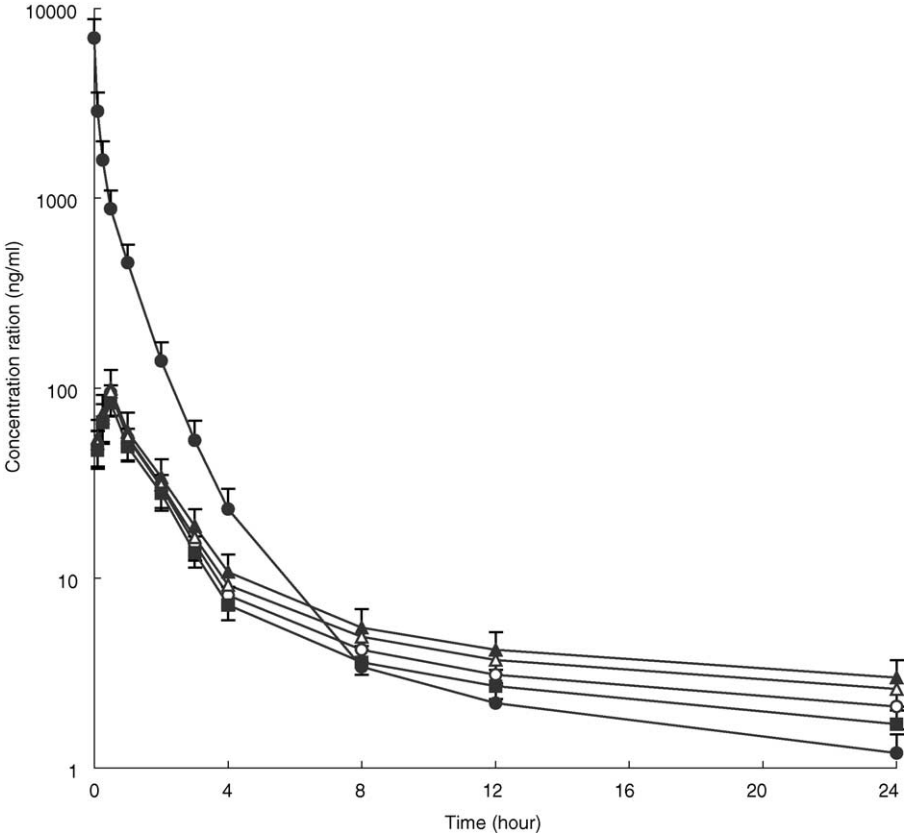

Fig. 1. Mean plasma concentration-time profiles of diltiazem after the oral co-administration of diltiazem (15 mg/kg) with quercetin to the rabbits. Bars represent the standard deviation ($n = 8$), (◯) diltiazme control, (▲) co-administered with quercetin 2 mg/kg, (Δ) co-administered with quercetin 10 mg/kg, (■) co-administered with quercetin 20 mg/kg; (●) diltiazem i.v. 5 mg/kg.

Table 2
Pharmacokinetic parameters of diltiazem after the oral administration of diltiazem (15 mg/kg) pretreated with quercetin to the rabbits

Parameters	Diltiazem control	Quercetin pre-treatment (mg/kg)			i.v. (5 mg/kg)
		2	10	20	
AUC (ng/ml h)	232 ± 58	$406 \pm 107^{*}$	$566 \pm 142^{**}$	$642 \pm 166^{**}$	1669 ± 467
C_{max} (ng/ml)	94.2 ± 23.5	$164.4 \pm 43.4^{*}$	$252.1 \pm 63.0^{**}$	$262.1 \pm 68.0^{**}$	
T_{max} (h)	0.5	0.5	0.5	0.5	
K_{el} (h^{-1})	0.061 ± 0.022	0.060 ± 0.019	0.054 ± 0.024	0.054 ± 0.010	0.124 ± 0.031
$t_{1/2}$ (h)	11.3 ± 2.8	11.5 ± 3.0	12.8 ± 3.2	12.8 ± 3.3	5.6 ± 1.4
AB%	4.64 ± 0.64	$9.10 \pm 1.21^{*}$	$11.29 \pm 1.98^{**}$	$12.81 \pm 2.17^{**}$	100
RB%	100	175	224	276	

Mean ± S.D. ($n = 8$), $^{*}p < 0.05$, $^{**}p < 0.01$, significant difference compared to control; AUC: area under the plasma concentration-time curve from 0 to 24 h; C_{max}, peak concentration; T_{max}, time to reach peak concentration; K_{el} (h^{-1}), elimination rate constant; $t_{1/2}$, terminal half-life; RB%, AUC rate compared to $AUC_{control}$; AB%, absolute bioavailability.

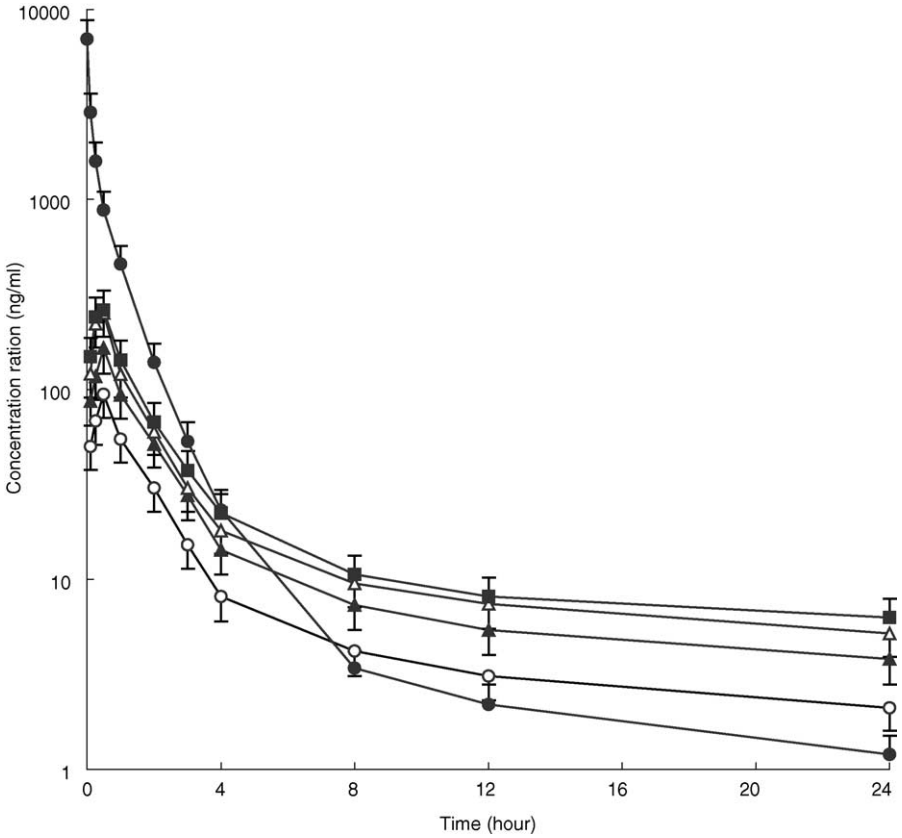

Fig. 2. Mean plasma concentration-time profiles of diltiazem after the oral administration of diltiazem (15 mg/kg) pretreated with quercetin to the rabbits; bars represent the standard deviation ($n = 8$), (◯) diltiazme control, (▲) pretreated with quercetin 2 mg/kg, (△) pretreated with quercetin 10 mg/kg, (■) pretreated with quercetin 20 mg/kg, (●) dltiazem i.v. 5 mg/kg.

inhibited by P-gp efflux pump (Yusa and Tsuruo, 1989; Saeki et al., 1993). P-gp and CYP 3A4 are believed to act synergistically to the first-pass metabolism. The increased AUCs, and C_{max} of diltiazem by pretreatment of quercetin might have been resulted from the inhibition of the P-gp efflux pump and the metabolizing enzyme, CYP 3A4, in the intestinal mucosa. Quercetin could inhibit the P-gp efflux pump (Scambia et al., 1994; Shapiro and Ling, 1997) and restrain the metabolizing enzyme, CYP3A4 (Dupuy et al., 2003; Miniscalco et al., 1992; Guengerich and Kim, 1990). Therefore, it might increase the bioavailability of diltiazem by inhibiting CYP 3A4 to reduce the metabolism and inhibiting P-gp to increases the absorption through the intestinal mucosa. This is consistent with the report by Wang et al. (2004), in that the co-administration of quercetin (digoxin administered after quercetin) significantly increased the C_{max} and AUC of digoxin (substrate for P-gp and CYP 3A4) in pigs. It is also consistent with Zhang et al. (2000), in that the pretreatment with naringin led to increase in C_{max} and AUC of quinine, the substrate of CYP 3A4 and P-gp, but no significant difference in $t_{1/2}$.

The bioavailibility of diltiazem pretreated with quercetin is increased significantly compared with the control, but not by co-administration of quercetin. By co-administration of quercetin (2, 10, 20 mg/kg), the AB% of diltiazem is decreased dose-dependently from 5.73 to 4.21, especially by co-administration of the high dose of quercetin, 20 mg/kg. Hsiu et al. (2002) reported that significantly decreased cyclosporin AUC by oral concomitant administration of quercetin (50 mg/kg) with Cycolosporin, a substrate for CYP 3A4 and P-gp, to pigs and rats, on the other hand, shown significant

inhibition of P-gp function by quercetin at the everted intestine sac. It might be considered that the quercetin interact with diltiazem in the gastrointestinal lumen to form the complex by co-administration of the high dose of quercetin, or the absorption of quercetin in the gastrointestinal mucosa is early enough to inhibit diltiazem metabolizing enzyme CYP3A4 and efflux pump P-gp by pretreatment of quercetin 30 min before diltiazem, but not by co-administration.

Although it is need to investigate further, it appears that diltiazem dose, which is widely used in clinics, should be taking into consideration when diltiazem is administered with diets or supplements containing quercetin over a long period. As flavonoids like quercetin are widely distributed in the daily diet as glycosides, it is necessary to investigate the effect of flavonoid glycosides on the bioavailability of many drugs act as substrate for CYP 3A4 and P-gp in the future study.

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