ORIGINAL ARTICLES

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Effects of epigallocatechin gallate on the bioavailability and pharmacokinetics of diltiazem in rats

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This study investigated the effect of orally administered epigallocatechin gallate (EGCG), a flavonoid, on the bioavailability or pharmacokinetics of diltiazem and its main active metabolites desacetyldiltiazem in rats. Pharmacokinetic parameters of diltiazem and desacetyldiltiazem were determined following the oral administration of diltiazem ($15\ mg \cdot kg^{-1}$) in the presence or absence of EGCG (1, 4 and $12\ mg \cdot kg^{-1}$). The area under the plasma concentration-time curve (AUC) and the peak concentration (C_{max}) of diltiazem were significantly ($P < 0.05$, $4\ mg \cdot kg^{-1}$ and $P < 0.01$, $12\ mg \cdot kg^{-1}$) increased in the presence of EGCG. The total body clearance (CL/F) was significantly ($P < 0.05$, 4 and $12\ mg \cdot kg^{-1}$) decreased in the presence of EGCG. Consequently, the absolute bioavailability (AB%) of diltiazem was significantly ($P < 0.05$, $4\ mg \cdot kg^{-1}$ and $P < 0.01$, $12\ mg \cdot kg^{-1}$) increased compared with that of the control group. The relative bioavailability (RB%) of diltiazem was from 1.65- to 1.76-fold higher than that of the control group. The terminal half-life ($t_{1/2}$) and time to reach the peak concentration (T_{max}) of diltiazem did not change significantly in the presence of EGCG. EGCG significantly ($P < 0.05$, $12\ mg \cdot kg^{-1}$) increased the AUC and C_{max} of desacetyldiltiazem. Metabolite-parent AUC ratio of desacetyldiltiazem was decreased, but did not change significantly. The presence of EGCG significantly enhanced the oral bioavailability of diltiazem due to inhibiting cytochrome P450 (CYP) 3A4-mediated metabolism and P-glycoprotein (P-gp) mediated efflux of diltiazem in the intestine.

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; Weir 1995). Diltiazem undergoes an extensive first-pass metabolism, and the absolute bioavailability is approximately 40%, with a large inter-individual variation. In humans and dogs, *N*-demethyldiltiazem is the most abundant metabolite in plasma. In contrast, desacetyldiltiazem and *O*-deacetyl-*N*-monodemethyldiltiazem were most predominant in rabbits and rats, respectively (Buckley et al. 1990; Yeung et al. 1998). The hypotensive potency of desacetyldiltiazem (active metabolite) is estimated to be about one half that of diltiazem (Buckley et al. 1990; Yeung et al. 1998; Narita et al. 1986). Cytochrome P450 (CYP) 3A4 is the main metabolizing enzyme for diltiazem, which is mainly located in the liver and in the small intestine (Kolars et al. 1992; Pichard et al. 1990; Watkins et al. 1987). Thus, diltiazem could be metabolized in small intestine as well as in liver (Homsy et al. 1995a; 1995b; Lefebvre et al. 1996). Lee et al. (1991) have reported that the extraction ratios of diltiazem in small intestine and liver were about 85 and 63% in rats, respectively.

In addition to the extensive metabolism, P-glycoprotein (P-gp) may also account for the low bioavailability of diltiazem. Yusa and Tsuruo (1989) reported that the calcium channel blockers such as verapamil, nicardipine and diltiazem competitively restrain the multi-drug resistance of P-gp. Wacher et al. (2001) reported that diltiazem was substrate to both CYP3A4 and P-gp. In the small intestine, P-gp is co-localized at the apical membrane of the cells with CYP3A4 (Gottesman and Pastan 1993). P-gp and CYP3A4 might act synergistically to the first-pass metabolism (Lefebvre et al. 1996; Gan et al. 1996; Wacher et al. 1998; Watkins 1996).

Flavonoids represent a group of phytochemicals which are produced by various plants in high quantities (Dixon and Steele 1999). They exhibit a wide range of beneficial biological activities including antioxidative, radical scavenging, antiatherosclerotic, antitumor and antiviral effects (Nijveldt et al. 2001).

Epigallocatechin gallate (EGCG) is the major flavanoid component in green tea and is also known as catechin (Chu and Juneja 1997). EGCG has a wide range of biological and pharmacological activities, including antioxidant (Higdon and Frei 2003), antimutagenic and anticarcinogenic activities (Kuroda and Hara 1999). EGCG inhibited human CYP3A4 with IC_{50} value of 10 μM (Muto et al. 2001), and has an inhibitory effect on P-gp in human Caco-2 cells (Jodoin et al. 2002). Hong et al. (2003) reported that EGCG and its methyl metabolites are sub-

strates for MRP1 and MRP2 but not for P-gp. EGCG is also subject to UDP-glucuronosyltransferase, sulfotransferase and catechol-*O*-methyltransferase mediated phase II biotransformation (Lu et al. 2003). In the present study, EGCG inhibited the P-gp substrates, diltiazem in KB-C2 cell (Kitagawa et al. 2004). Green tea is a widely consumed beverage in many countries, mainly in Korea, China and Japan. A prospective cohort study of a Japanese population revealed that the daily intake of EGCG in green tea in these subjects was calculated to be 500–700 mg (Muto et al. 2001), which was about 10 mg · kg^{-1} body weight.

As a dual inhibitor of CYP3A4 and P-gp, EGCG might affect the pharmacokinetics of diltiazem. However, the effect of EGCG on the *in vivo* pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, has not been reported. This study focused on the investigation of the effect of EGCG on the pharmacokinetics of diltiazem and its main metabolites, desacetyldiltiazem, in rats.

2. Investigations and results

The plasma concentration-time profiles of diltiazem after oral administration of diltiazem (15 mg · kg^{-1}) in presence or absence of EGCG (1, 4 and 12 mg · kg^{-1}) are shown in Fig. 1. The pharmacokinetic parameters of diltiazem are summarized in Table 1. As shown in Table 1, the presence of EGCG significantly ($P < 0.05$, 4 mg · kg^{-1} and $P < 0.01$, 12 mg · kg^{-1}) increased the peak concentration (C_{max}) and the areas under the plasma concentration-time curve (AUC) of diltiazem compared to those of the control group. Total body clearance was decreased significantly ($P < 0.05$, 4 and 12 mg · kg^{-1}) by EGCG. Consequently, the absolute bioavailability (AB%) of diltiazem was significantly ($P < 0.05$, 4 mg · kg^{-1} and $P < 0.01$, 12 mg · kg^{-1}) increased compared with that of the control group. Relative bioavailability (RB%) of diltiazem in the presence of EGCG was 1.65- to 1.76-fold times higher. The terminal half-life ($t_{1/2}$) and time to reach the peak concentration (T_{max}) of diltiazem were not altered significantly in the presence of EGCG.

The plasma concentration-time profiles of deacetyldiatiazem are shown in Fig. 2. The pharmacokinetic parameters of deacetyldiatiazem were summarized in Table 2. AUC and C_{max} of desacetyldiltiazem were increased significantly ($p < 0.05$, 12 mg · kg^{-1}) with EGCG. The metabolite-parent ratio (MR; AUC of desacetyldiltiazem to diltiazem) of desacetyldiltiazem in the rats coadministered with

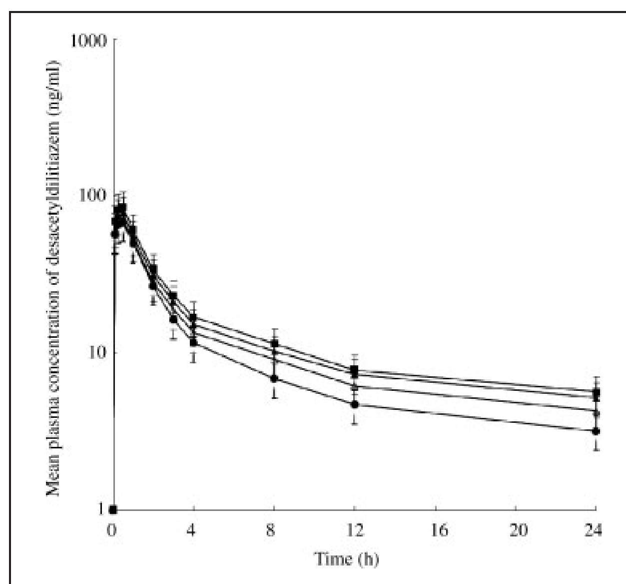

Fig. 2: Mean plasma concentration-time profiles of desacetyldiltiazem after the oral (15 mg · kg^{-1}) administration of diltiazem to rats in the presence or absence of EGCG (Mean ± SD, n = 6). The bar represent the standard deviation, ●, Control (diltiazem 15 mg · kg^{-1} oral); △, the presence of 1 mg · kg^{-1} of EGCG; ▲, the presence of 4 mg · kg^{-1} of EGCG; ■, the presence of 12 mg · kg^{-1} of EGCG

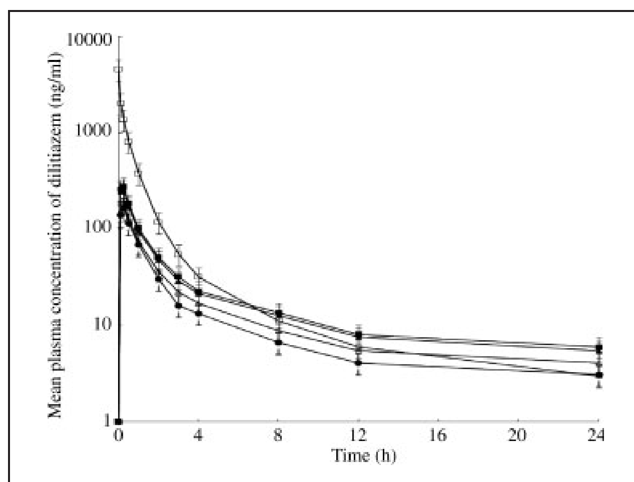

Fig. 1: Mean plasma concentration-time profiles of diltiazem after the intravenous (5 mg · kg^{-1}) or oral (15 mg · kg^{-1}) administration of diltiazem to rats in the presence or absence of EGCG (Mean ± SD, n = 6). The bar represent the standard deviation, ●, Control (diltiazem 15 mg · kg^{-1} oral); △, the presence of 1 mg · kg^{-1} of EGCG; ▲, the presence of 4 mg · kg^{-1} of EGCG; ■, the presence of 12 mg · kg^{-1} of EGCG; □, intravenous administration of diltiazem 5 mg · kg^{-1}

Table 1: Mean pharmacokinetic parameters of diltiazem following the intravenous (5 mg · kg^{-1}) or oral (15 mg · kg^{-1}) administration of diltiazem to rats in the presence or absence of EGCG (mean ± SD, n = 6)

Parameters	Diltiazem (Control)	Diltiazem + EGCG			i.v. (5 mg · kg^{-1})
		1 mg · kg^{-1}	4 mg · kg^{-1}	12 mg · kg^{-1}	
AUC/ng · h · mL^{-1}	352 ± 73.9	431 ± 89.7	580 ± 112	619 ± 126	1749 ± 439
C_{max}/ng · mL^{-1}	165 ± 34.6	189 ± 44.2	260 ± 58.9	272 ± 61.4**	
T_{max}/h	0.25	0.25	0.25	0.25	
CL/F/mL · min^{-1} · kg^{-1}	709 ± 178	580 ± 139	431 ± 111	403 ± 100	
$t_{1/2}$/h	10.2 ± 2.71	10.5 ± 2.83	11.1 ± 2.90	11.4 ± 2.94	6.4 ± 1.57
AB/%	6.71 ± 1.31	8.22 ± 1.86	11.1 ± 2.92	11.9 ± 3.04	100
RB/%	100	122	165	176	–

* $P < 0.05$, ** $P < 0.01$ compared to control. $AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; CL/F: total body clearance; $t_{1/2}$: terminal half-life; AB: absolute bioavailability; RB: relative bioavailability

Table 2: Mean pharmacokinetic parameters of desacetyldiltiazem, a major metabolite of diltiazem following the oral administration of diltiazem (15 mg · kg^{-1}) to rats in the presence or absence of EGCG (mean ± SD, n = 6)

Parameters	Diltiazem (Control)	Diltiazem + EGCG		
		1 mg · kg^{-1}	4 mg · kg^{-1}	12 mg · kg^{-1}
AUC/ng · h · mL^{-1}	294 ± 63.6	358 ± 81.4	418 ± 91.2*	453 ± 107*
C_{max}/ng · mL^{-1}	68.1 ± 14.0	71.2 ± 17.5	78.3 ± 19.1	85.4 ± 20.4*
T_{max}/h	0.5	0.5	0.5	0.5
$t_{1/2}$/h	11.6 ± 1.89	12.8 ± 2.38	13.2 ± 2.39	13.5 ± 3.11
MR	0.84 ± 0.19	0.83 ± 0.20	0.72 ± 0.17	0.71 ± 0.16

* $P < 0.05$ compared to control. $AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; $t_{1/2}$: terminal half-life; MR: $AUC_{desacetyldiltiazem}/AUC_{diltiazem}$.

4 and 12 mg · kg^{-1} of EGCG decreased by approximately 20% compared to the control group, but the metabolite-parent ratios was not signicantly changed in the presence of EGCG. The terminal half-life ($t_{1/2}$) and time to reach the peak concentration (T_{max}) of desacetyldiltiazem were not altered significantly in the presence of EGCG.

3. Discussion

The presence of EGCG significantly enhanced the oral bioavailability of diltiazem by increasing intestinal absorption as well as reducing the first-pass metabolism. Based on the broad overlap in the substrate specificities as well as co-localization in the small intestine, the primary site of absorption for orally administered drugs, CYP3A4 and P-gp have been recognized as a main barrier to the drug absorption (Cummins et al. 2002).
The oral bioavailability of diltiazem is approximately 40% due to extensive first-pass metabolism through CYP3A4 mediated *N*-demethylation and desacetylation (Buckley et al. 1990; Pichard et al. 1990; Choi and Li 2005). CYP3A4 is mainly located in the liver, and in the intestine (Kolars et al. 1992; Watkins et al. 1987). Therefore, metabolism of diltiazem mainly involves both the liver and the small intestine (Homsy et al. 1995a, 1995b; Lefebvre et al. 1996). The other important factor interfering oral diltiazem absorption is P-gp, a MDR efflux transporter. Diltiazem appeared to be a substrate of P-gp (Wacher et al. 2001; Saeki et al. 1993). Therefore, CYP3A4 and P-gp may act synergistically to limit the oral bioavailability of diltiazem (Lefebvre et al. 1996; Saeki et al. 1993). With the great interest in herbal products of alternative medicine, much effort is currently being expanded toward identifying natural compounds from plants that modulate P-gp as well as metabolic enzymes. Therefore, more preclinical and clinical studies on the herbal constituents-drug interaction should be performed to prevent potential adverse reactions or to utilize those interactions for a therapeutic benefit. Thus, the present study evaluated the effect of EGCG, antioxidant and flavonoid, on the pharmacokinetics of diltiazem in rats to examine a potential drug interaction between EGCG and diltiazem via the dual inhibition of CYP3A4 and P-gp.
In this study, as summarized in Table 1, the presence of EGCG significantly increased the C_{max} and AUC of diltiazem. These results were consistent with those of Homsy et al. (1995a) and Muto et al. (2001) in that EGCG was a inhibitor of CYP3A4. Presence of EGCG significantly increased the bioavailability of diltiazem. These results were consistent with those of Saeki et al. (1993) and Kitagawa et al. (2004) in that EGC-G inhibited P-gp in human Caco-2 cells (Jodoin et al. 2002), and in KB-C2 cell. The enhanced oral bioavailability of diltiazem might be due to the decreased efflux and metabolism of diltiazem in the intestine and liver, which presents P-gp and CYP3A. These results were similar to those of Choi et al. (2005a, b) in that flavonoids (morin and naringin), an inhibitor of CYP3A and P-gp, significantly enhanced the oral bioavailability of diltiazem in rats and rabbits, respectively.
The pharmacokinetic parameters of desacetyldiltiazem were evaluated in the presence of EGCG (Table 2). Presence of EGCG significantly increased the AUC and C_{max} of desacetyldiltiazem. The presence of EGCG decreased the metabolite-parent ratio of desacetyldiltiazem but the effect was not significant. These results were different from those of Choi and Han (2005), where the presence of morin could inhibit effectually metabolite-parent ratio of diltiazem in rats.
On the whole, the bioavailability of diltiazem was significantly enhanced in the presence of EGCG (4 and 12 mg · kg^{-1}), a dual inhibitor of CYP3A4 and P-gp. This result further confirmed the suggestion that EGCG enhances the bioavailability of diltiazem through inhibition of CYP3A4 and P-gp. The importance of these findings require further investigation in clinical trials.

4. Experimental

4.1. Materials

Diltiazem hydrochloride, desacetyldiltiazem, EGCG and imipramine (an internal standard for HPLC analysis of diltiazem and desacetyldiltiazem) were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). Saline (0.9% NaCl injectable solution) was obtained from Choongwae Co. (Seoul, Republic of 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 microcentrifuge (National Labnet, USA) and a sonicator (Daihan Co., Republic of Korea).

4.2. Animal studies

Male Sprague-Dawley rats (280–300 g) were purchased from Dae Han Laboratory Animal Research and Co. (Eumsung, Republic of Korea), and had free access to normal standard chow diet (Superfeed Company, Wonju, Republic of Korea) and tap water. Throughout the experiment, the animals were housed, four or five per cage, in laminar flow cages maintained at 22 ± 2 °C, 50–60% relative humidity, under a 12 h light-dark cycle. The animals were kept in these facilities for at least one week before the experiment. This experiment was 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 in our institution (Chosun University) approved the present study.
Rats were divided into five groups (n = 6, each): a control group (diltiazem 15 mg · kg^{-1}, oral), co-administration groups (15 mg · kg^{-1} diltiazem co-administered orally with EGCG 1, 4 and 12 mg · kg^{-1}, respectively) and an intravenous group (5 mg · kg^{-1} diltiazem dissoved in a 0.9% NaCl-injectable solution). Both diltiazem and EGCG were dissolved in 1.2 mL distilled water just before given by gavage. A feeding tube was used to

administer diltiazem and EGCG orally. EGCG was administered 30 min prior to diltiazem. For i.v. group, the diltiazem injectable solution ($5\ mg \cdot kg^{-1}$) was injected through the femoral vein within 0.5 min. The rats were fasted for at least 24 h prior to experiments and were given water freely. The right femoral artery (for blood sampling) and the femoral vein (for drug administration for intravenous administration) were cannulated with polyethylene tube (PE-50, Intramedic, Clay Adams, NJ, USA). Blood samples (0.5 mL) were collected into heparinized tubes via the femoral artery at 0 (to serve as a control), 0.017 (only for i.v. administration), 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 h after the administering the diltiazem. The blood samples were immediately centrifuged at 13,000 rpm for 5 min and a 0.2 mL aliquot of the plasma samples was stored in a $-40\ ^{\circ}C$ until HPLC analysis of diltiazem and desacetyldiltiazem.

4.3. HPLC Assay

The plasma concentrations of diltiazem were determined by a HPLC assay by modification of the method reported by Goebel and Kolle (1985). Briefly, a 50 μL of imipramine ($2\ \mu g \cdot mL^{-1}$), as the internal standard, and a 1.2 mL of *tert*-butylmethylether were added to a 0.2 mL of the plasma sample. It was then mixed for 2 min using a vortex-mixer and centrifuged at 13,000 rpm for 10 min. The organic layer (1 mL) was transferred to another clean test tube, 0.2 mL of 0.01 N hydrochloride was added and mixed for 2 min. 50 μL of the water layer were injected into the HPLC system.

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) was used as the mobile phase at a flow rate of $1.5\ mL \cdot min^{-1}$. The retention times were as follows: internal standard at 10.5-min, diltiazem at 8.0-min and desacetyldiltiazem at 6.5 min. The detection limit of diltiazem and desacetyldiltiazem in plasma was $5\ ng \cdot mL^{-1}$. The intra- and inter-day variation coefficients of diltiazem and desacetyldiltiazem were both below 5%.

4.4. Pharmacokinetic analysis

The plasma concentration data were analyzed using a noncompartmental method on WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The terminal half-life ($t_{1/2}$) 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 the plasma were obtained by a visual inspection of the data from 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 infinite ($AUC_{0-\infty}$) was obtained by adding AUC_{0-t} and the extrapolated area was determined by C_{last}/K_{el}. The total body clearance (CL/F) was calculated from the dose/AUC. The absolute bioavailability (AB%) was calculated by ($AUC_{oral}/AUC_{i.v.} \times dose_{i.v.}/dose_{oral}) \times 100$, and the relative bioavailability (RB%) was calculated by $AUC_{control}/AUC_{with\ EGCG} \times 100$. The metabolite-parent ratio (MR) was estimated from ($AUC_{desacetyldiltiazem}/AUC_{diltiazem}) \times 100$.

4.5. Statistical analysis

All mean values are presented with their standard deviation (Mean ± S.D.). Statistical analysis was conducted using a one-way ANOVA followed by *a posteriori* testing with Dunnett's correction. Differences were considered significant at a level of $p < 0.05$

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