[UCI]I804:24011-200000236462

2008 년 8 월

박사학위논문

DRUG INTERACTION BETWEEN PIOGLITAZONE OR METFORMIN AND DILTIAZEM IN RATS

조선대학교 대학원

약학과

이 일 권

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흰쥐에서 피오글리타존 및 메트폴민과 딜티아젬의 약물동태학적 상호작용

2008 년 8 월 25 일

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지도교수 최 준 식

이 논문을 약학박사학위신청 논문으로 제출함.

2008 년 4 월

조선대학교 대학원

약학과

이 일 권

이일권의 박사학위논문을 인준함

위원장	서울대학교	교수	이명걸	인
위 원	전남대학교	교수	신상철	인
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2008 년 8 월

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Drug Interaction Between Pioglitazone or Metformin with Diltiazem in Rats

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Diltiazem, a substrate of cytochrome P450 (CYP) 3A and P-glycoprotein (P-gp), is a calcium channel blocker that is widely used for the treatment of angina, supraventricular arrhythmias and hypertension. The present study aimed to investigate the effect of pioglitazone or metformin on the pharmacokinetics of diltiazem in rats. Pharmacokinetic parameters of diltiazem were determined in rats after oral administration of diltiazem (15 mg/kg) in the presence and absence of pioglitazone (1 or 3 mg/kg) or metformin (10 or 30 mg/kg).

The presence of pioglitazone significantly (1 mg/kg, $P < 0.05$; 3 mg/kg, $P < 0.01$) increased the area under the plasma concentration–time curve (AUC) of diltiazem. Pioglitazone significantly ($P < 0.05$) increased the peak concentration (C_{max}) of diltiazem. Pioglitazone increased the terminal half-life ($t_{1/2}$) of diltiazem but not significantly. Consequently, the relative bioavailability (RB) of diltiazem was increased by 1.49– to 1.68–fold than those of the control group. Pioglitazone increased the terminal half-life ($t_{1/2}$) of diltiazem but not significantly. It did not change the peak concentration time (T_{max}) of diltiazem. The enhanced bioavailability of diltiazem might be due to the decreased first-pass metabolism (CYP3A) of diltiazem in the intestine and/or liver competitively.

The presence of 3 mg/kg of pioglitazone significantly (P <0.05) increased the area under the plasma concentration–time curve (AUC) of desacetyldiltiazem. Pioglitazone increased the peak plasma concentration (C_{max}) and the terminal half-life ($t_{1/2}$) of desacetyldiltiazem but not significantly. Consequently, the relative bioavailability (RB) of desacetyldiltiazem increased by 1.32– to 1.48–fold than those of the control group.

The presence of metformin, antioxidant, significantly altered the pharmacokinietic parameters of diltiazem. Compared to the oral control group (given diltiazem alone), the presence (10 and 30 mg/kg) of metformin significantly (P < 0.05) increased the area under the plasma concentration–time curve ($AUC_{0-\infty}$) and peak plasma concentration (C_{max}) of diltiazem. Consequently, the relative bioavailability (RB) of diltiazem was increased approximately 1.34– to 1.54–fold in the presence of metformin. Metformin increased the terminal half-life ($t_{1/2}$) of diltiazem but not significantly. It did not change in the peak concentration time (T_{max}) of diltiazem.

The presence of 30 mg/kg of metformin significantly (P <0.05) increased the area under the plasma concentration–time curve (AUC) of desacetyldiltiazem. Metformin increased the peak plasma concentration (C_{max}) and the terminal half-life ($t_{1/2}$) of desacetyldiltiazem but not significantly. Consequently, the relative bioavailability (RB) of desacetyldiltiazem increased by 1.26– to 1.41–fold than those of the control group. Metformin increased the terminal half-life ($t_{1/2}$) of desacetyldiltiazem but not significantly. It did not change the peak concentration time (T_{max}) of desacetyldiltiazem.

The presence of pioglitazone or metformin significantly enhanced the oral bioavailability of diltiazem. Based on the results, the diltiazem dosage adjustment should be taken into consideration for safe and effective therapy of hypertension disease with diabetic complication when diltiazem is used concomitantly with pioglitzone or metformin in the clinical setting.

Key words: Diltiazem, Desacetyldiltiazem, Pioglitazone, Metformin, CYP3A, P-gp, Pharmacokinetics, Bioavailability, Rats

국 문 초 록

흰쥐에서 피오글리타존 또는 메트폴민과 딜티아젬의 약물동태학적인 상호작용

이 일 권

지 도 교 수: 최 준 식

조선대학교대학원 약학과

딜티아젬 (diltiazem)은 CYP 3A와 P-당단백질의 기질이며 칼슘채널을 차단하여 협심증과 고혈압치료에 널리 사용된다. 따라서, 본 실험에서는 흰쥐에게 딜티아젬 (15 mg/kg)과 피오글리타존 (pioglitazone, 1 혹은 10 mg/kg) 혹은 메트폴민 (metformin, 10 혹은 30 mg/kg)을 동시에 경구투여하였을 때 딜티아젬 및 그 활성대사체인 데스아세틸딜티아젬의 약물동태학적파라미터를 연구검토하였다. 흰쥐를 2개의 실험군으로 분류하였으며 제1실험군은 딜티아젬 단독 경구(15 mg/kg) 투여하였으며 제2실험군은 피오글리타존 (1 및 3 mg/kg) 혹은 메트폴민 (10 및 30 mg/kg)을 각각 딜티아젬과 동시에 경구투여 하였다.

피오글리타존과 동시투여하였을 때 딜티아젬의 약물동태학적 파라미터는 유의성 있게 변화하였다. 대조군에 비해 피오글리타존 동시투여군에서 딜티아젬의 혈장농도곡선하면적 ($AUC_{0-\infty}$)은 유의성 (1 mg/kg, $p<0.05$; 3 mg/kg, $p<0.01$) 있게 증가하였으며 최고혈중농도 (C_{max})도 유의성 ($p<0.05$) 있게 증가하였다. 상대생체이용률 (RB)은 대조군에 비해 1.49-1.68 배로 증가하였다. 피오글리타존

동시투여군에서 소실반감기 ($t_{1/2}$)는 대조군에 비해 길어졌으나 유의성은 없었다. 피오글리타존 동시투여군에서 최고 혈중농도 도달시간 (T_{max})는 유의성은 없었다.

대조군에 비해 피오글리타존 3 mg/kg 을 동시투여하였을 때 데스아세틸딜티아젬의 혈장농도곡선하면적 ($AUC_{0-\infty}$)은 유의성 ($p < 0.05$) 있게 증가하였다. 상대생체이용률 (RB)은 1.32-1.48 배로 증가하였다. 피오글리타존 동시투여군에서 데스아세틸딜티아젬의 최고혈중농도 (C_{max}), 소실반감기 ($t_{1/2}$) 및 최고 혈중농도 도달시간 (T_{max})은 유의성 있는 변화가 없었다.

딜티아젬과 메트폴민 (항산화제)을 동시투여하였을 때 딜티아젬의 약물동태학적 파라미터는 유의성 있게 변화하였다. 대조군에 비해 메트폴민 (10 및 30 mg/kg) 동시투여군에서 딜티아젬의 혈장농도곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 유의성 ($p < 0.05$) 있게 증가하였다. 상대생체이용률 (RB)은 대조군에 비해 1.34-1.54 배로 증가하였다. 대조군에 비해 메트폴민 동시투여군에서 소실반감기 ($t_{1/2}$)는 증가하였으나 유의성 있는 변화가 없었으며 최고혈중농도 도달시간 (T_{max})는 유의성 있게 변화가 없었다.

대조군에 비해 메트폴민 동시투여군에 비해 데스아세틸딜티아젬의 혈장농도곡선하면적 ($AUC_{0-\infty}$)은 유의성 ($p < 0.05$) 있게 증가하였다. 상대생체이용률 (RB)은 대조군에 비해 1.26-1.41 배로 증가하였다. 대조군에 비해 메트폴민 동시투여군에서 최고혈중농도 (C_{max}) 및 소실반감기 ($t_{1/2}$)는 증가하였으나 유의성 있는 변화거 없었으며 최고혈중농도 도달시간 (T_{max})는 유의성 있게 변화가 없었다.

본 연구에서 당뇨병 치료제인 피오글리타존 및 메트폴민을 각각 고혈압치료제인 딜티아젬과 동시투여 하였을 때 경구투여시킨 딜티아젬의 생체이용률은 현저히 증가시켰다. 본 연구결과를 토대로,

임상에서 고혈압을 동반한 당뇨병환자에 두가지 약물을 함께 투여할 때 약물간에 상호작용을 연구검토하는 것이 바람직하다고 사료된다.

Part I. Drug Interaction Between Pioglitazone and Diltiazem in Rats

Abstract

Diltiazem, a substrate of cytochrome P450 (CYP) 3A and P-glycoprotein (P-gp), is a calcium channel blocker that is widely used for the treatment of angina, supraventricular arrhythmias and hypertension. This study investigated the effect of pioglitazone on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem in rats. A single dose of diltiazem was administered orally (15 mg/kg) without or with pioglitazone (1 or 3 mg/kg).

The presence of pioglitazone significantly (1 mg/kg, $P < 0.05$; 3 mg/kg, $P < 0.01$) increased the area under the plasma concentration–time curve (AUC) of diltiazem. Pioglitazone significantly ($P < 0.05$) increased the peak concentration (C_{max}) of diltiazem. Pioglitazone increased the terminal half-life ($t_{1/2}$) of diltiazem but not significantly. Consequently, the relative bioavailability (RB) of diltiazem increased by 1.49– to 1.68–fold than those of the control group. Pioglitazone increased the terminal half-life ($t_{1/2}$) of diltiazem but not significantly, and did not change the peak concentration time (T_{max}) of diltiazem. The enhanced bioavailability of diltiazem might be due to the decreased first-pass metabolism (CYP3A4) of diltiazem in the intestine and/or liver competitively.

The presence of 3 mg/kg of pioglitazone significantly ($P < 0.05$) increased the area under the plasma concentration–time curve (AUC) of desacetyldiltiazem. Pioglitazone increased the peak plasma concentration (C_{max}) and the terminal half-life ($t_{1/2}$) of desacetyldiltiazem but not significantly. Consequently, the relative bioavailability (RB) of desacetyldiltiazem increased by 1.32– to 1.48–fold than those of the control group.

The presence of pioglitazone significantly enhanced the oral bioavailability of diltiazem. Based on the results, the diltiazem dosage adjustment should be taken into consideration for safe and effective therapy of hypertension disease with diabetic complecation when diltiazem is used concomitantly with pioglitzone in the clinical setting.

Key words: Diltiazem, Desacetyldiltiazem, Pioglitazone, CYP3A, Pharmacokinetics, Bioavailability, Rats.

1. Introduction

Diltiazem, (2*S*,3*S*)-5-[2-(dimethyl-amino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate (Figure 1), is a calcium channel blocker. The drug is widely used for the treatment of angina, supraventricular arrhythmias and hypertension (Chaffman *et al*., 1985; Yeung *et al*., 1993; Weir, 1995). Diltiazem undergoes extensive and complex phase I metabolized to desacetylation, N-demethylation, and O-demethylation. Its extent of absolute oral bioavailability (F) is approximately 40%, with a large inter-subject variability (Buckley *et al*., 1990; Yeung *et al*., 1993). Based on the preclinical studies, the estimated hypotensive potency of desacetyldiltiazem appeared to be about one-half of diltiazem, whereas that of N-demethyldiltiazem and N-demethyldesacetyl-diltiazem was about one-third of diltiazem (Narita *et al*., 1986; Yeung *et al*., 1998). Considering the potential contribution of the active metabolites to the therapeutic outcome of diltiazem treatment, it coul be important to monitor the active metabolites as well as the parent drug in the pharmacokinetic studies of diltiazem. Diltiazem is subjected to extensive intestinal metabolism by different microsomal cytochrome P 450 (CYP) 3A4 and esterases, and this reduces the amount of unchanged diltiazem into the portal by over 50% of the oral dose (Lee *et al*., 1991; Lefebvre *et al*., 1996; Molden *et al*., 2000; Iwao *et al*., 2004) (Figure 1). Deacetylation of diltiazem, a main metabolic pathway in rats` intestine (Lee *et al*., 1991), is mediated via esterases (LeBoeuf and Grech-Belanger, 1987). CYP3A4, a key enzyme for the metabolism of diltiazem in humans is mainly located in the liver, but it is also expressed in the small intestine (Watkins *et al*., 1987; Pichard *et al*., 1990; Kolars *et al*., 1992). Thus, diltiazem could be metabolized in the small intestine as well as in liver (Homsy *et al*., 1995a; Homsy *et al*., 1995b; Lefebvre et al., 1996). Lee *et al*. (1991) reported that the extraction ratios of diltiazem in the small intestine and the liver after oral administration to rats were about 85 and 63%,

respectively, suggesting that diltiazem is highly extracted in the small intestine. Yusa and Tsuruo (1989) reported that the calcium channel blockers such as verapamil and diltiazem competitively restrained the multi-drug resistance of P-glycoprotein (P-gp). Wacher *et al*. (2001) suggested that diltiazem is a substrate of both CYP3A4 and P-gp. Since P-gp is co-localized with CYP3A4 in the small intestine. Thus P-gp and CYP3A4 may act synergistically for the absorption of presystemic metabolism of drug, respectively (Gottesman and Pastan, 1993; Gan *et al*., 1996; Wacher *et al*., 1998; Ito *et al*., 1999; Wacher *et al*., 2001).

A peroxisome proliferator–activated receptor (PPAR) γ, a member of the nuclear receptor superfamily of ligand-activated transcription, plays a crucial role in adipogenesis and insulin resistance. A PPAR γ is highly expressed in adipose tissue, and is also exists in myocytes, vascular smooth muscle cells, and macrophages/monocytes, as well as in adipocytes (Mukherjee *et al*., 1997 Schiffrin *et al*., 2003) Thiazolidinediones, which are PPAR γ activators, improve insulin sensitivity and are used as an anti-diabetic drug. This category of drug has also been shown to exert anti-inflammatory and anti-fibrotic avtivites in animal models of cardiovascular diseases, including atherosclerosis, vascular inflammation, and cardiac failure (Schiffrin *et al*., 2003; Mukherjee *et al*., 1997; Ishibashi *et al*., 2002).

Pioglitazone, (Figure 2) a thiazolidinedione antidiabetic drug that increases insulin sensitivity in target tissues (Chilcott *et al*., 2001), has F value of 80%, and is metabolized via multiple cytochrome P450 (CYP) isoenzymes, mainly by CYP2C8, CYP3A4 and CYP2C9 to several active and inactive metabolites (Gillies *et al*., 2000). A recent report suggests that rifampicin; an inducer of CYP3A4 decreased the total area under the plasma concentration–time curve from time zero to time infinity (AUC) of pioglitazone by 35% whereas, gemfirozil; an inhibitor of CYP2C8 increased the AUC by 239% (Scheen, 2007). *In vitro*, pioglitazone has been reported to inhibit both CYP2C8 (Sahi *et al*., 2003; Walsky *et al*., 2005; Kajosaari *et al*., 2006) and CYP3A4 enzymes (Sahi *et al*., 2003; Kajosaari *et al*.,

2006). But the inhibitory effects of the pioglitazone have not been demonstrated *in vivo* (Kajosaari *et al*., 2006). These evidences suggest that pioglitazone can be a prime candidate for several drug–drug interactions, particularly because large number of drugs is reported to modulate the CYP450 enzyme activity.

The multiple prescription of drug is increasingly common in current medical practice, and anti-diabetic agent could be coadministered with drugs used for the treatment of hypertension such as calcium channel blockers in patients. But there is less report about their interactions between diltiazem and pioglitazone *in vivo*. Thus, this study was performed to investigate the effect of pioglitazone on the pharmacokinetics of diltiazem and its main metabolism, desacetyldiltiazem, in rats.

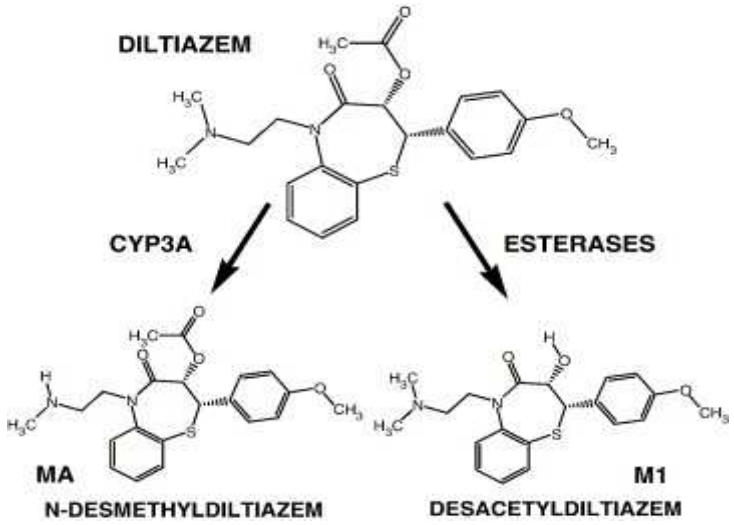

Figure 1. Main metabolite pathways of DTZ in rats.

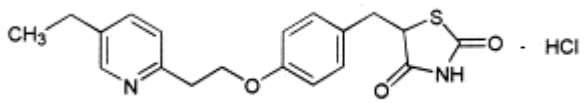

Figure 2. Structure of pioglitazone hydrochloride.

2. Materials and methods

2.1. Chemicals

Diltiazem, desacetyldiltiazem, pioglitazone and imipramine [internal standard to the high-performance liquid chromatographic (HPLC) analysis of diltiazem and desacetyldiltiazem] were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Methanol and acetonitrile (HPLC grade) were produced from Merck Co. (Darmstadt, Germany). Other chemicals were of reagent grade or HPLC grade.

2.2. Drug administration

The protocols of the animal studies were approved by the Animal Care Committee of Chosun University (Gwangju, Republic of Korea). Male Sprague–Dawley rats (7–8 weeks of age is weighing 270 to 300 g) were purchased from the Dae Han Laboratory Animal Research Co. (Eumsung, Republic of Korea), and were given access to a normal standard chow diet (No. 322-7-1) purchased from the Superfeed Co. (Wonju, Republic of Korea) and tap water *ad libitum*. Throughout the experiment, the animals were housed, four or five per cage, in laminar flow cages maintained at 22 ± 2^{o}C, and 50–60% relative humidity, under a 12:12 h light-dark cycle throughout the experiment.

The rats were randomly divided into three groups (n = 6, each): oral administration of diltiazem at a dose of 15 mg/kg without or with oral administration of pioglitazone at a dose of 1 or 3 mg/kg. The rats were fasted for at least 24 h prior to beginning of the experiments. Each animal was anaesthetized with ether and the right femoral artery (for blood sampling) was cannulated with a polyethylene tube (Clay Adams, Parsippany, N.J.).

The diltiazem solution was diluted in distilled water to make a 15 mg/kg. The

pioglitazone was suspended in distilled water. Blood samples (0.5 ml) were collected into heparinized tubes via the femoral artery at 0 (to serve as a control), 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, 8, 12 and 24 h after the oral administration of diltiazem. Blood samples were centrifuged (13,000 rpm, 5 min), and the plasma sample was stored at –40℃ until use for the HPLC analysis of diltiazem and desacetyldiltiazem.

2.3. HPLC analysis of diltiazem and desacetyldiltiazem

2.3.1. Sample preparation

Plasma concentrations of diltiazem were determined using a slight modified of the reported HPLC assay Goebel *et al*. (1985). Briefly, 50 μl of imipramine (2 μg/ml; internal standard), and 1.2 ml of tert-butylmethylether were added to a 0.2 ml of samples. The mixture was then stirred for 2 min and centrifuged for (13,000 rpm, 10 min). One ml of the organic layer was transferred to a clean test tube and 0.2 ml of 0.01 N hydrochloride was added and mixed for 2 min. 50 μl of the water layer was injected into the HPLC system.

2.3.2. HPLC condition

The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu, Japan), a UV-Vis detector (Model SPD-10A), a system controller (Model SCL-10A), a degasser (Model DGU-12A) and an autoinjector (SIL-10AD). The mobile phase was methanol : acetonitrile : 0.04 M ammonium bromide : triethylamine (24 : 31 : 45 : 0.1, v/v/v, pH 7.4, adjusted with acetic acid) was run to flow rate of 1.5 ml/min, and the column [μ-bondapack C_{18} column (3.9 mm, i.d. × 300 mm, 10 μm) Waters Co., Ireland] eluent was monitored at 237 nm at room

temperature. The retention times of desacetyldiltiazem, diltiazem and internal standard was 6.9-min, 8.7-min and 9.7-min, respectively (Figure 3). The calibration curves of diltiazem and desacetyldiltiazem were linear within the ranges of 5–500 ng/ml (Figures 4 and Figures 5). The intra- and inter-day (n = 5) coefficients of variation were less than 5% for diltiazem and desacetyldiltiazem, respectively. Detection limit of diltiazem and desacetyldiltiazem was 10 ng/ml

2.4. Pharmacokinetic analysis

The following pharmacokinetic data were analyzed using the non-compartmental method (WinNonlin software version 4.1; Pharsight Corporation, Mountain View, CA, USA). The half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and the time to reach peak concentration (t_{max}) of diltiazem or desacetyldiltiazem directly read from the experimental data.. The area under the plasma concentration time-curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area was determined by C_{last}/K_{el}. The relative bioavailability (RB) was estimated by $AUC_{coadmin}/AUC_{control} \times 100$. The metabolite-parent ratio (MR) was estimated by $AUC_{desacetyldiltiazem}/AUC_{diltiazem}$

2.5. Statistical analysis

Statistical analysis was conducted using a one-way analysis of viariance (ANOVA) followed by *a posteriori* testing with Dunnett correction using the means for the unpaired data. Differences were deemed be significant at a level of $p < 0.05$. All data were expressed in terms of the mean ± S.D.

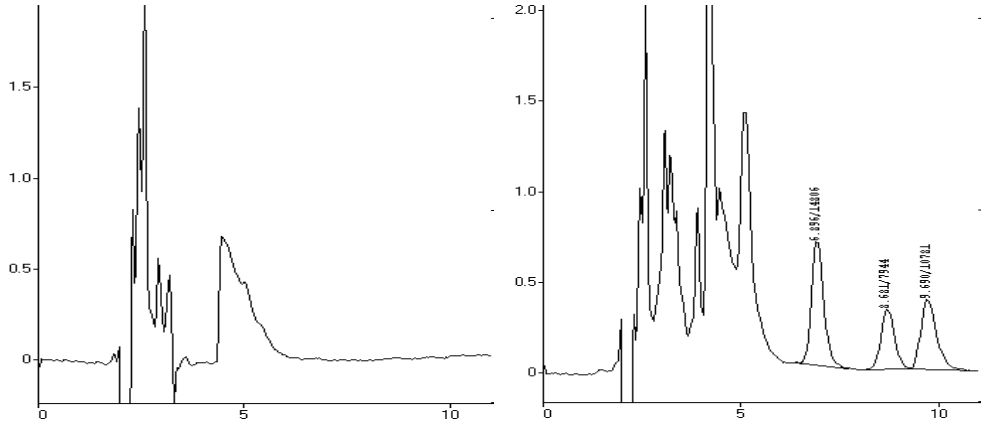

Figure 3. HPLC chromatograms of the rat's blank plasma (A), and the plasma spiked with diltiazem (8.7 min), desacetyldiltiazem (6.9 min), and imipramine (internal standard; 9.7 min) (B).

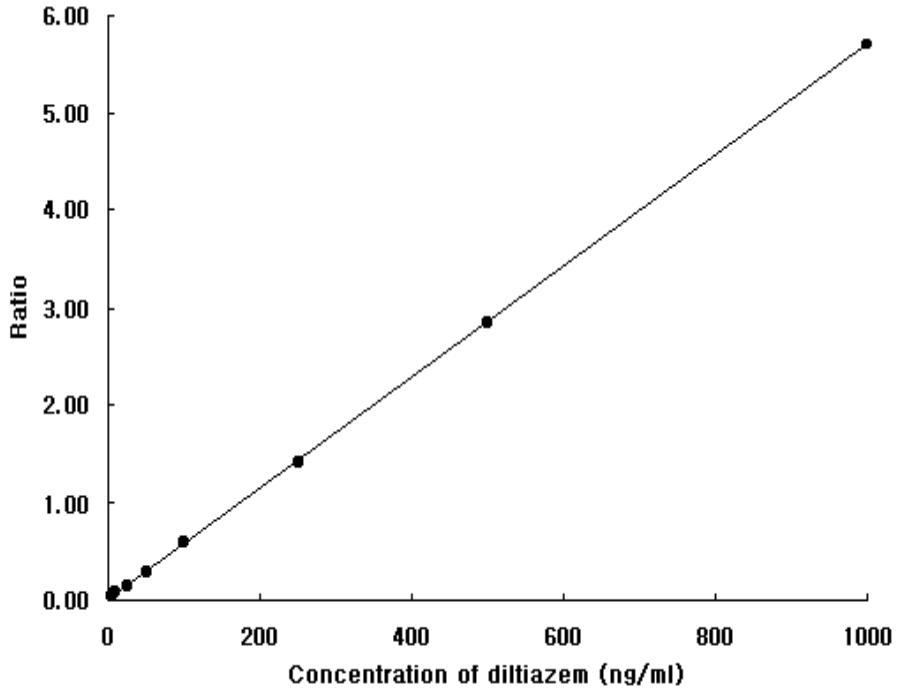

Figure 4. A calibration curve of diltiazem when spiked into the rat's blank plasma.

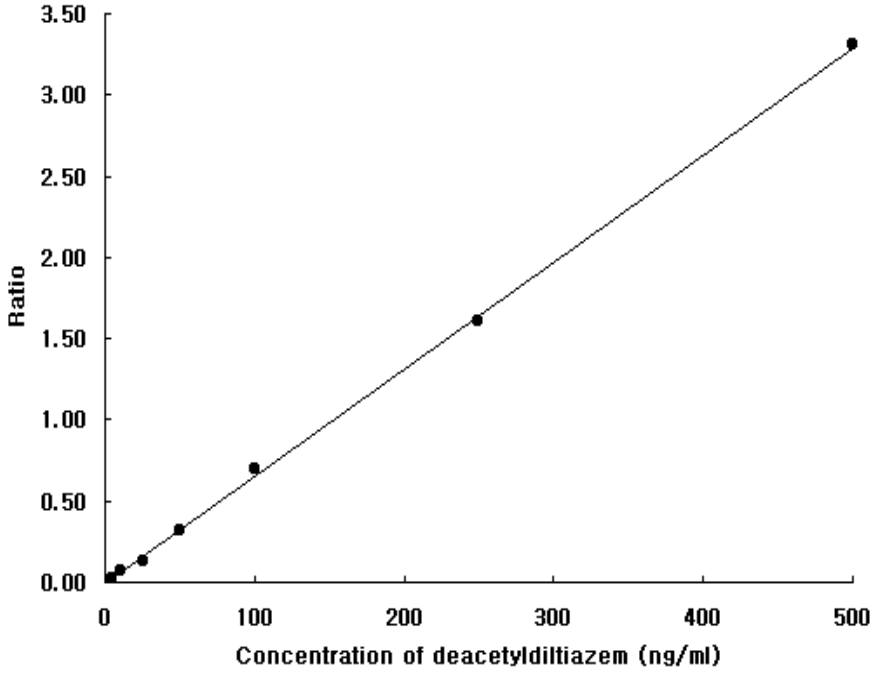

Figure 5. A calibration curve of desacetyldiltiazem when spiked into the rat's blank plasma.

Table 1. Mean (± S.D.) plasma concentrations of diltiazem after oral administration of diltiazem (15 mg/kg) without or with pioglitazone to rats (*n* = 6, each).

Time (h)	Control (Diltiazem)	Diltiazem + Pioglitazone	
		1 mg/kg	3 mg/kg
0	0	0	0
0.1	138 ± 33.1	203 ± 48.6	219 ± 52.6
0.25	162 ± 38.8	242 ± 58.6	266 ± 63.7
0.5	112 ± 26.8	158 ± 37.8	171 ± 41.0
1	67 ± 16.1	86 ± 20.5	94.3 ± 22.6
2	29.5 ± 7.07	42.6 ± 10.2	47.5 ± 11.4
3	16.7 ± 4.01	27 ± 6.45	30.4 ± 7.29
4	13.6 ± 3.24	19.7 ± 4.71	21.5 ± 5.16
8	7.0 ± 1.67	11.2 ± 2.89	12.4 ± 2.97
12	4.2 ± 1.0	6.4 ± 1.6	7.5 ± 1.8
24	3.1 ± 0.73	5.0 ± 1.2	5.7 ± 1.3

Table 2. Mean (± S.D.) plasma concentrations of desacetyldiltiazem after oral administration of diltiazem (15 mg/kg) with or without of pioglitazone in rats (*n* = 6, each).

Time (h)	Control (Diltiazem)	Diltiazem + Pioglitazone	
		1 mg/kg	3 mg/kg
0	0	0	0
0.1	55.6 ± 13.4	59.2 ± 14.3	63.4 ± 15.2
0.25	64.4 ± 15.3	71.8 ± 17.2	77.5 ± 18.1
0.5	66.1 ± 15.7	74.6 ± 18.1	80.0 ± 19.3
1	48.7 ± 11.6	52.8 ± 12.7	56.7 ± 13.6
2	26.0 ± 6.24	30.1 ± 7.22	32.3 ± 7.75
3	15.7 ± 3.76	20.2 ± 4.85	21.7 ± 5.21
4	11.3 ± 2.71	14.5 ± 3.67	16.5 ± 3.96
8	6.7 ± 1.61	9.7 ± 2.3	10.6 ± 2.54
12	4.6 ± 1.1	6.8 ± 1.6	7.4 ± 1.8
24	3.1 ± 7.4	4.7 ± 1.2	5.4 ± 1.3

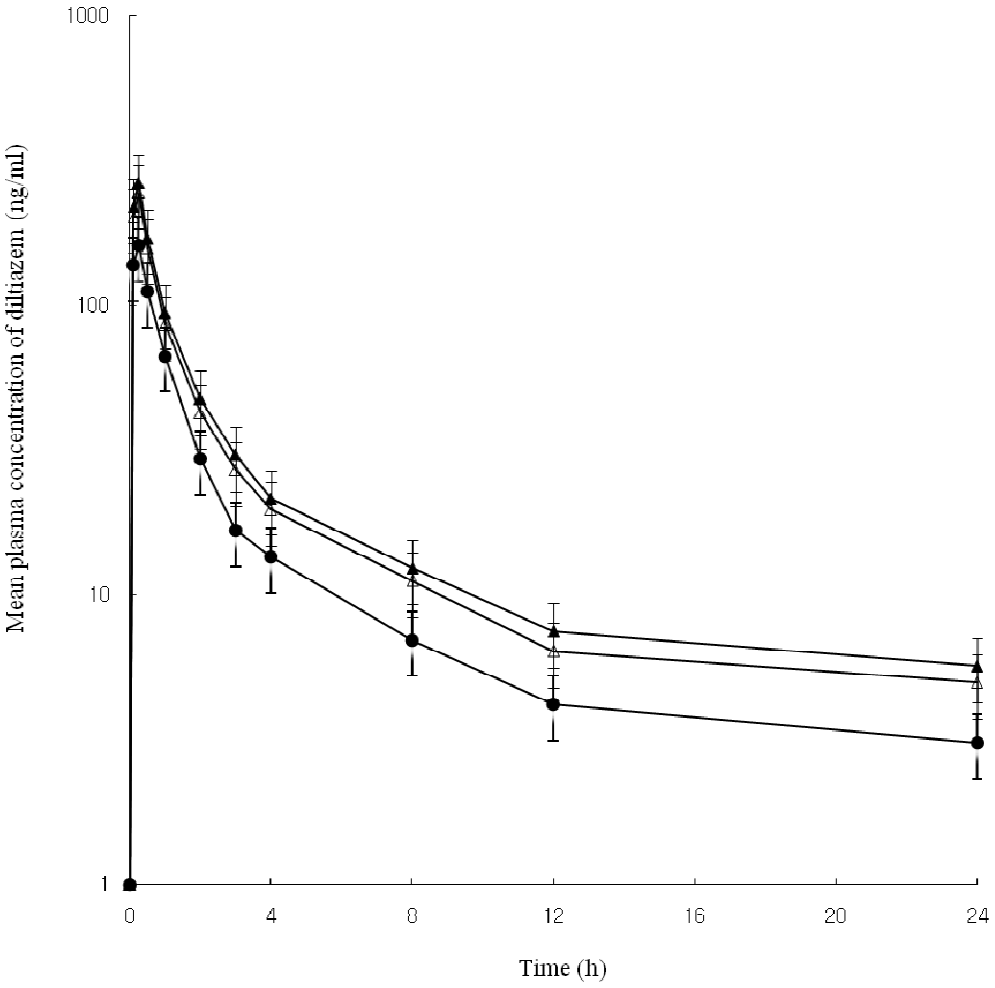

Figure 6. Mean arterial plasma concentration–time profiles of diltiazem after oral administration of diltiazem (15 mg/kg) without (●) or with 1 mg/kg (△) or 3 mg/kg (▲) of pioglitazone to rats (*n* = 6, each). Bars represent the standard deviation.

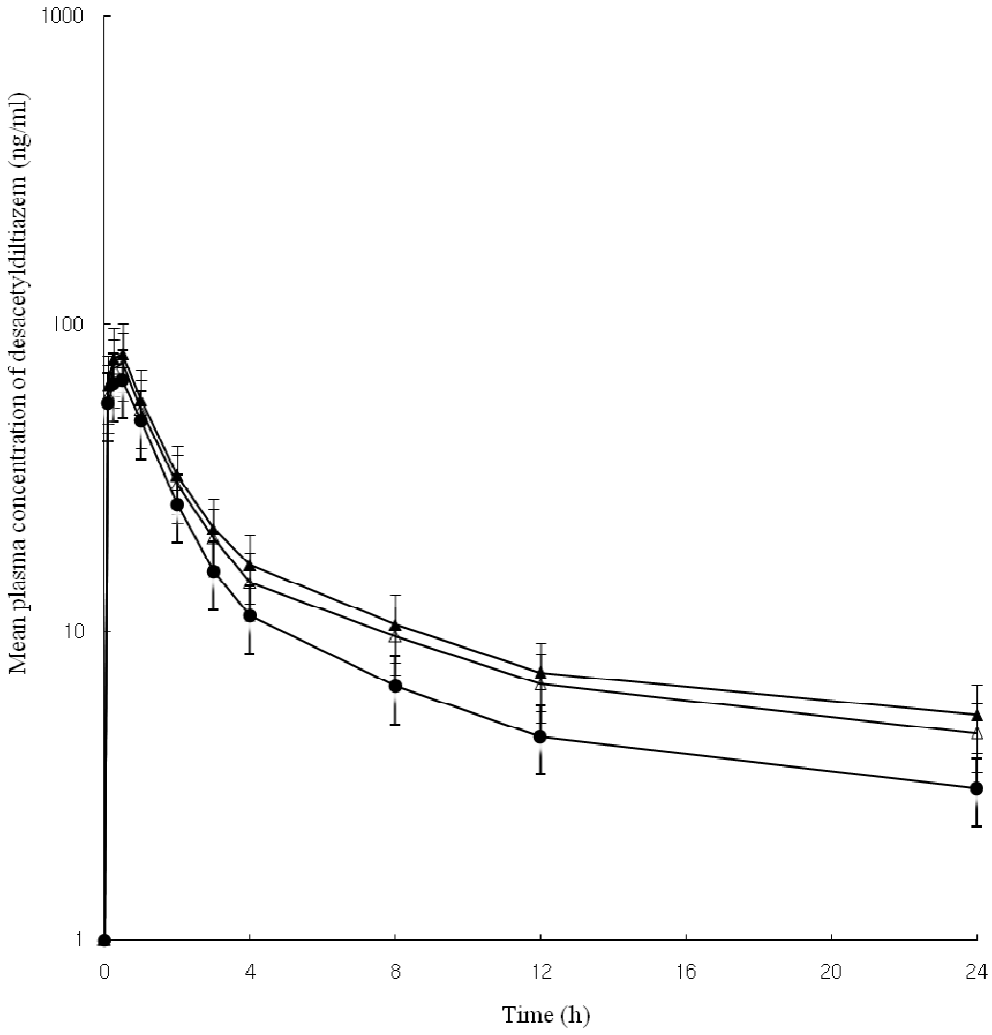

Figure 7. Mean arterial plasma concentration–time profiles of desacetyldiltiazem after oral administration of diltiazem (15 mg/kg) without (●) or with 1 mg/kg (△) or 3 mg/kg (▲) of pioglitazone to rats (*n* = 6, each). Bars represent the standard deviation.

Table 3. Mean (± S.D.) pharmacokinetic parameters of diltiazem after oral administration of diltiazem (15 mg/kg) with or without of pioglitazone to rats (*n* = 6, each).

Parameter	Control	Diltiazem + Pioglitazone	
		1 mg/kg	3 mg/kg
AUC (ng·h/ml)	348 ± 83.5	518 ± 125*	583 ± 139**
C_{max} (ng/ml)	162 ± 38.9	242 ± 58.8*	266 ± 63.8*
T_{max} (h)	0.25	0.25	0.25
$t_{1/2}$ (h)	10.3 ± 2.47	10.9 ± 2.62	11.3 ± 2.83
RB (%)	100	149	168

* P < 0.05, **P < 0.01 compared to control.

AUC: area under the plasma concentration–time curve from time 0 time to infinity;

C_{max}: peak plasma concentration;

T_{max}: time to reach C_{max};

$t_{1/2}$: terminal half-life;

RB: relative bioavailability.

Table 4. Mean (± S.D.) Pharmacokinetic parameters of desacetyldiltiazem after oral administration of diltiazem (15 mg/kg) with or without of pioglitazone to rats (*n* = 6, each).

Parameter	Control	Diltiazem + Pioglitazone	
		1 mg/kg	3 mg/kg
AUC (ng·h/ml)	285 ± 68.4	384 ± 95.6	424 ± 102*
C_{max} (ng/ml)	66.1 ± 15.8	74.6 ± 18.0	80.0 ± 19.3
T_{max} (h)	0.5	0.5	0.5
$t_{1/2}$ (h)	11.6 ± 2.78	12.8 ± 3.12	13.1 ± 3.16
RB (%)	100	132	148
MR	0.82 ± 0.18	0.74 ± 0.17	0.73 ± 0.17

* $P < 0.05$ 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;

RB: relative bioavailability;

MR: metabolite-parente ratio.

3. Results and Discussion

Figure 3 shows the HPLC chromatograms of the rat's blank plasma (A) and the plasma spiked with diltiazem, desacetyldiltiazem and imipramine (internal standard) (B). The retention of diltiazem, desacetyldiltiazem and imipramine was 8.7, 6.7 and 9.7 min, respectively.

The calibration curves of diltiazem (Figure 4) and desacetyldiltiazem (Figure 5) were linear within the concentration ranges from 5–500 ng/ml, respectively. The detection limits for diltiazem and descetyldiltiazem was 5 ng/ml. The intra- and inter-day (n = 5) coefficients of variation were less than 5% for diltiazem and desacetyldiltiazem.

Figure 6 shows the mean plasma concentration–time profiles of diltiazem after oral administration (9 mg/kg) with or without of pioglitazone (1 or 3 mg/kg), and Table 3 lists the relevant pharmacokinetic parameters of diltiazem after oral administration. Pioglitazone significantly (1 mg/kg, $P < 0.05$; 3 mg/kg, $P < 0.01$) increased the area under the plasma concentration–time curve (AUC) of diltiazem. Pioglitazone significantly ($P < 0.05$) increased the peak concentration (C_{max}) of diltiazem. Pioglitazone increased the terminal half-life ($t_{1/2}$) of diltiazem but not significantly. Consequently, the relative bioavailability (RB) of diltiazem increased by 1.49– to 1.68–fold than those of the control group. CYP3A4, a key enzyme for the metabolism of diltiazem is mainly located in liver, and in small intestine (Watkins *et al*., 1987; Pichard *et al*., 1990; Kolars *et al*., 1992). The pharmacokinetic studies indicated that pioglitazone is metabolized by multiple cytochrome P450 (CYP) isoenzymes, mainly by CYP3A4 and CYP2C8 to several active and inactive metabolites (Gillies *et al*., 2000). *In vitro*, pioglitazone has been reported to inhibit CYP3A4 enzymes (Sahi *et al*., 2003; Kajosaari *et al*., 2006). The enhanced bioavailability of diltiazem by pioglitazone might be due to the

competitive inhibition of CYP3A4. This result appeared to be consistent with previous studies reported by Hong et al. (2007) and Choi *et al*. (2006); a single oral administration of atorvastatin and fluvastatin significantly increased the AUC and C_{max} of diltiazem in rats, respectively, which was due to inhibition of CYP3A4. Pioglitazone did not significantly chage the T_{max} of diltiazem.

Figure 7 depicts the mean plasma concentration–time profiles of desacetyldiltiazem after oral administration of diltiazem (15 mg/kg) with or without pioglitazone (1 or 3 mg/kg). As listed in Table 4, 3 mg/kg of pioglitazone significantly (P <0.01) increased the area under the plasma concentration–time curve (AUC) of desacetyldiltiazem. Pioglitazone increased the peak plasma concentration (C_{max}) and the terminal half-life ($t_{1/2}$) of desacetyldiltiazem but not significantly. Consequently, the relative bioavailability (RB) of desacetyldiltiazem increased by 1.32- to 1.48-fold than those of the control group. Compare to the control group, presence of pioglitazone (1 or 3 mg/kg) decreased the metabolite-parent ratio (MR) but not significantly. These results suggest the first-pass metabolism diltiazem might be inhibited by pioglitazone. Pioglitazone did not significantly chage the T_{max} of desacetyldiltiazem.

CYPs in enterocytes contribute significantly to the “first-pass” metabolism and oral bioavailability of many drugs and chemicals. The “first pass” metabolism of compounds in the intestine limits absorption of toxic xenobiotics and may ameliorate adverse effects. Moreover, induction or inhibition of intestinal CYPs may be responsible for significant drug/drug interactions when one agent decreases or increases the F and K_a of biotransformation of a concurrently administered drug (Kaminsky and Fasco, 1991).

The increased bioavailability of diltiazem by pioglitazone suggests that CYP3A could be competitively inhibited by pioglitazone, which resulted in reducing first-pass metabolism of diltiazem in the intestine and/or liver. The adjustment of the dose of diltiazem should be taken into consideration for potential interaction

between pioglitazone and diltiazem in clinical setting.

4. Conclusion

Presence of pioglitazone significantly enhanced the systemic bioavailability of diltiazem in rats. If the results are further confirmed in the clinical trial, dose adjustment of diltiazem shoud been taken into consideration when diltiazem is treated with concomitantly with pioglitazone to the patients.

References

Buckley MMT, Grant SM, Goa KL, McTabish D, Sorkin EM. Diltiazem: a reappraisal of its pharmacological properties and therapeutic use. Drugs. 1990; 39: 757–806.

Chaffman M, Brogden RN. Diltiazem: a review of its pharmacological properties and therapeutic efficacy. Drugs. 1985; 29: 387–454.

Chilcott J, Tappenden P, Jones ML, Wight JP. A systematic review of the clinical effectiveness of pioglitazone in the treatment of type 2 diabetes mellitus. Clin Ther. 2001; 23: 1792–1823.

Choi HJ, Choi JS. Effects of morin pretreatment on the pharmacokinetics of diltiazem and its major metabolite, desacetyldiltiazem in rats. Arch Pharm Res. 2005; 28: 970–976.

Choi JS, Han HK. Pharmacokinetic interaction between diltiazem and morin, a flavonoid, in rats. Pharmacol Res. 2005; 52: 386–391.

Choi JS, Li X. Enhanced diltiazem bioavailability after oral administration of diltiazem with quercetin to rabbits. Int J Pharm. 2005; 297: 1–8.

Choi JS, Piao YJ, Han HK. Pharmacokinetic interaction between fluvastatin and diltiazem in rats. Biopharm Drug Dispos. 2006; 27: 437–441.

Gan LSL, Moseley MA, Khosla B, Augustijns PF, Bradshaw TP, Hendren RW, Thakker DR. CYP3A-Like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells: interaction between the two biochemical barriers to intestinal transport. Drug Metab Dispos. 1996; 24: 344–349.

Gillies PS, Dunn CJ. Pioglitazone. Drugs. 2000; 60: 333–343.

Goebel KJ, Kolle EU. High performance liquid chromatographic dertermination of diltiazem and four of its metabolites in plasma. J Chromatogr. 1985; 345: 355–363.

Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Ann Rev Biochem. 1993; 62: 385–427.

Homsy W, Caille G, du Souich P. The site of absorption in the small intestine determines diltiazem bioavailability in the rabbit. Pharm Res. 1995a; 12: 1722–1726.

Homsy W, Lefebvre M, Caille G, du Souich P. Metabolism of diltiazem in hepatic and extrahepatic tissues of rabbits: *in vitro* studies. Pharm Res. 1995b; 12: 609–614.

Hong SP, Chang KS, Choi DH, Choi JS. Effect of atorvastatin on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats. Arch Pharm Res. 2007; 30: 90–95.

Ishibashi M, Egashira K, Hiasa K. Antiinflammatory and antiarteriosclerotic effects of pioglitazone. Hypertension. 2002; 40: 687–693.

Ito K, Kusuhara H, Sugiyama Y. Effects of intestinal CYP3A4 and P-glycoprotein on oral drug absorption; theoretical approach. Pharm Res. 1999; 16: 225–231.

Iwao T, Inoue K, Hayashi Y, Yuasa H, Watanabe J. Absorption and metabolic extraction of diltiazem from the perfused rat small intestine, Drug Metab Pharm. 2004; 19: 430–437.

Kajosaari LI, Jaakkola T, Neuvonen PJ, Backman JT. Pioglitazone, an *in vitro* inhibitor of CYP2C8 and CYP3A4, does not increase the plasma concentrations of the CYP2C8 and CYP3A4 substrate repaglinide. Eur J Clin Pharmacol. 2006; 62: 217–223.

Kaminsky LS, Fasco MJ. Small intestinal cytochromes P450. Crit Rev Toxicol. 1991; 21: 407–422.

Kolars JC, Schmiedlin-Ren P, Dobbins WO, Schuetz J, Wrighton SA, Watkins PB. Heterogeneity of cytochrome P450IIIA expression in rat gut epithelia. Gastroenterology. 1992; 102: 1186–1198.

LeBoeuf E, Grech-Belanger O. Deacetylation of diltiazem by rat liver. Drug Metab

Dispos. 1987; 15: 122–126.

Lee YH, Lee MH, Shim CK. Pharmacokinetics of diltiazem and deacetyldiltiazem in rats. Int J Pharm. 1991; 76: 71–76.

Lefebvre M, Homsy W, Caille G, du Souich P. First-pass metabolism of diltiazem in anesthetized rabbits: role of extrahepatic organs. Pharm Res. 1996; 13: 124–128.

Molden E, Christensen H, Sund RB. Extensive metabolism of diltiazem and P-glycoprotein-mediated efflux of desacetyl-diltiazem (M1) by rat jejunum *in vitro*. Drug Metab Dispos. 2000; 28: 107–109.

Mukherjee R, Jow L, Croston GE, Paterniti JR. Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARγ2 versus PPARγ1 and activation with retinoid X receptor agonists and antagonists. J Biol Chem. 1997; 272: 8071–8076.

Narita H, Otsuka M, Yabana H, Nagao T. Hypotensive response of spontaneously hypertensive rats to centrally administered diltiazem and its metabolites: in relevance to the hypotensive action by oral administration. J Pharmacobiodyn. 1986; 9: 547–553.

Pichard L, Gillet G, Fabre I, Dalet-Beluche I, Bonfils C, Thenot JP, Maurel P. Identification of the rabbit and human cytochromes P-450IIIA as the major enzymes involved in the N-demethylation of diltiazem. Drug Metab Dispos. 1990; 18: 711–719.

Sahi J, Black CB, Hamilton GA, Zheng X, Jolley S, Rose KA, Gilbert D, LeCluyse EL, Sinz MW. Comparative effects of thiazolidinediones on *in vitro* P450 enzyme induction and inhibition. Drug Metab Dispos. 2003; 31: 439–446.

Scheen AJ. Pharmacokinetic interactions with thiazolidinediones. Clin Pharmacokin. 2007; 46: 1–12.

Schiffrin EL, Amiri F, Benkirane K, Iglarz M, Diep QN. Peroxisome proliferator−activated receptors: vascular and cardiac effects in hypertension. Hypertension. 2003; 42: 664–668.

Wacher VJ, Silverman JA, Zhang Y, Benet LZ. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. J Pharm Sci. 1998; 87: 1322–1330.

Wacher VJ, Salphati L, Benet LZ. Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv Drug Deliv Rev. 2001; 46: 89–102.

Walsky RL, Gaman EA, Obach RS. Examination of 209 drugs for inhibition of cytochrome P450 2C8. J Clin Pharmacol. 2005; 45: 68–78.

Watkins PB, Wrighton SA, Schuetz EG, Molowa DT, Guzelian PS. Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man. J Clin Invest. 1987; 80: 1029–1036.

Weir MR. Diltiazem: ten years of clinical experience in the treatment of hypertension. J Clin Pharmacol. 1995; 35: 220–232.

Yeung PK, Feng JDZ, Buckley SJ. Pharmacokinetics and hypotensive effect of diltiazem in rabbits: Comparison of diltiazem with its major metabolites. J Pharm Pharmacol. 1998; 50: 1247–1253.

Yeung PK, Prescott C, Haddad C, Montague TJ, McGregor C, Quilliam MA, Xei M, Li R, Farmer P, Klassen GA. Pharmacokinetics and metabolism of diltiazem in healthy males and females following a single oral dose. Eur J Drug Metab Pharmacokin. 1993; 18: 199–206.

Yusa K, Tsuruo T. Reversal mechanism of multidrug resistance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. Cancer Res. 1989; 49: 5002–5006.

Part II. Drug Interaction Between Metformin and Diltiazem in Rats

Abstract

Diltiazem, a substrate of cytochrome P450 (CYP) 3A and P-glycoprotein (P-gp), is a calcium channel blocker that is widely used for the treatment of angina, supraventricular arrhythmias and hypertension. This study investigated the effect of metformin on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem in rats. A single dose of diltiazem was administered orally (15 mg/kg) without or wity metformin (10 or 30 mg/kg)

The presence of metformin, antioxidant, significantly altered the pharmacokinietic parameters of diltiazem. Compared to the oral control group (given diltiazem alone), the presence of metformin (10 and 30 mg/kg) significantly ($P < 0.05$) increased the area under the plasma concentration–time curve ($AUC_{0-\infty}$) and peak plasma concentration (C_{max}) of diltiazem. Consequently, the relative bioavailability (RB) of diltiazem increased by approximately 1.34- to 1.54-fold in the presence of metformin. Metformin increased the terminal half-life ($t_{1/2}$) of diltiazem but not significantly, and did not change the peak concentration time (T_{max}) of diltiazem.

The presence of 30 mg/kg of metformin significantly ($P <0.05$) increased the area under the plasma concentration–time curve (AUC) of desacetyldiltiazem. Metformin increased the peak plasma concentration (C_{max}) and the terminal half-life ($t_{1/2}$) of desacetyldiltiazem but not significantly. Consequently, the relative bioavailability (RB) of desacetyldiltiazem increased by 1.26– to 1.41–fold than those of the control group. Metformin increased the terminal half-life ($t_{1/2}$) of desacetyldiltiazem but not significantly, and did not significantly change the peak

concentration time (T_{max}) of desacetyldiltiazem.

The presence of metformin significantly enhanced the oral bioavailability of diltiazem. Based on the results, the diltiazem dosage should be taken into consideration for safe and effective therapy of hypertension disease with diabetic complication when diltiazem is used concomitantly with metformin in the clinical setting.

Key words: Diltiazem, Desacetyldiltiazem, Metformin, CYP3A, Pharmacokinetics, Bioavailability, Rats.

1. Introduction

Diltiazem, (2*S*,3*S*)-5-[2-(dimethyl-amino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate (Figure 1), is a calcium channel blocker. The drug is widely used for the treatment of angina, supraventricular arrhythmias and hypertension (Chaffman *et al*., 1985; Yeung *et al*., 1993; Weir, 1995). Diltiazem undergoes extensive and complex phase I metabolized to desacetylation, N-demethylation, and O-demethylation. Its extent of absolute oral bioavailability (F) is approximately 40%, with a large inter-subject variability (Buckley *et al*., 1990; Yeung *et al*., 1993). Based on the preclinical studies, the estimated hypotensive potency of desacetyldiltiazem appeared to be about one-half of diltiazem, whereas that of N-demethyldiltiazem and N-demethyldesacetyl-diltiazem was about one-third of diltiazem (Narita *et al*., 1986; Yeung *et al*., 1998). Considering the potential contribution of the active metabolites to the therapeutic outcome of diltiazem treatment, it coul be important to monitor the active metabolites as well as the parent drug in the pharmacokinetic studies of diltiazem. Diltiazem is subjected to extensive intestinal metabolism by different microsomal cytochrome P 450 (CYP) 3A4 and esterases, and this reduces the amount of unchanged diltiazem into the portal by over 50% of the oral dose (Lee *et al*., 1991; Lefebvre *et al*., 1996; Molden *et al*., 2000; Iwao *et al*., 2004) (Figure 1). Deacetylation of diltiazem, a main metabolic pathway in rats` intestine (Lee *et al*., 1991), is mediated via esterases (LeBoeuf and Grech-Belanger, 1987). CYP3A4, a key enzyme for the metabolism of diltiazem in humans is mainly located in the liver, but it is also expressed in the small intestine (Watkins *et al*., 1987; Pichard *et al*., 1990; Kolars *et al*., 1992). Thus, diltiazem could be metabolized in the small intestine as well as in liver (Homsy *et al*., 1995a; Homsy *et al*., 1995b; Lefebvre et al., 1996). Lee *et al*. (1991) reported that the extraction ratios of diltiazem in the small intestine and the liver after oral administration to rats were about 85 and 63%,

respectively, suggesting that diltiazem is highly extracted in the small intestine. Yusa and Tsuruo (1989) reported that the calcium channel blockers such as verapamil and diltiazem competitively restrained the multi-drug resistance of P-glycoprotein (P-gp). Wacher *et al*. (2001) suggested that diltiazem is a substrate of both CYP3A4 and P-gp. Since P-gp is co-localized with CYP3A4 in the small intestine. Thus P-gp and CYP3A4 may act synergistically for the absorption of presystemic metabolism of drug, respectively (Gottesman and Pastan, 1993; Gan *et al*., 1996; Wacher *et al*., 1998; Ito *et al*., 1999; Wacher *et al*., 2001).

Metformin (Figure 8) is use for the treatment of type 2 diabetes as an adjunct to diet and exercise, either as a single oral agent or in combination with sulfonylureas, alpha-glucosidade inhibitors, or insulin (Setter *et al*., 2003; Robert *et al*., 2003). Metformin treatment is followed by improved oxidative stress, preserved antioxidant function, and restrained platelet activation in type 2 diabetic subjects (Gloria et al., 2008). Recently, it has been reported that metformin is mainly metabolized via the hepatic microsomal cytochrome P450 (CYP) 2C11, 2D1, and 3A1/2 in male Sprague–Dawley rats (Choi and Lee, 2006).

The multiple prescription of drug is increasingly common in current medical practice, and anti-diabetic agent could be coadministered with drugs used for the treatment of hypertension such as calcium channel blockers in patients. But there is less report about their interactions between diltiazem and metformin *in vivo*. Thus, this study was performed to investigate the effect of metformin on the pharmacokinetics of diltiazem and its main metabolism, desacetyldiltiazem, in rats.

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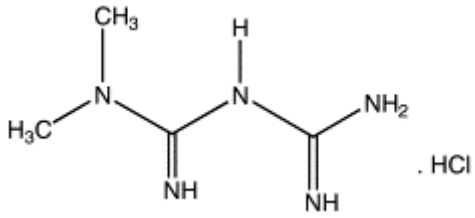

Figure 8. Chemical structure of metformin HCl.

2. Materials and methods

2.1. Chemicals

Diltiazem, desacetyldiltiazem, metformin and imipramine [internal standard to the high-performance liquid chromatographic (HPLC) analysis of diltiazem and desacetyldiltiazem] were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Methanol and acetonitrile (HPLC grade) were produced from Merck Co. (Darmstadt, Germany). Other chemicals were of reagent grade or HPLC grade.

2.2. Drug administration

The protocols of the animal studies were approved by the Animal Care Committee of Chosun University (Gwangju, Republic of Korea). Male Sprague–Dawley rats (7–8 weeks of age is weighing 270 to 300 g) were purchased from the Dae Han Laboratory Animal Research Co. (Eumsung, Republic of Korea), and were given access to a normal standard chow diet (No. 322-7-1) purchased from the Superfeed Co. (Wonju, Republic of Korea) and tap water *ad libitum*. Throughout the experiment, the animals were housed, four or five per cage, in laminar flow cages maintained at 22 ± 2^{o}C, and 50–60% relative humidity, under a 12:12 h light-dark cycle throughout the experiment.

The rats were randomly divided into three groups (n = 6, each): oral administration of diltiazem at a dose of 15 mg/kg without or with oral administration of metformin at a dose of 10 or 30 mg/kg. The rats were fasted for at least 24 h prior to beginning of the experiments. Each animal was anaesthetized with ether and the right femoral artery (for blood sampling) was cannulated with a polyethylene tube (Clay Adams, Parsippany, N.J.).

The diltiazem solution was diluted in distilled water to make a 15 mg/kg. The

metformin was suspended in distilled water. Blood samples (0.5 ml) were collected into heparinized tubes via the femoral artery at 0 (to serve as a control), 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, 8, 12 and 24 h after the oral administration of diltiazem. Blood samples were centrifuged (13,000 rpm, 5 min), and the plasma sample was stored at –40℃ until use for the HPLC analysis of diltiazem and desacetyldiltiazem.

2.3. HPLC analysis of diltiazem and desacetyldiltiazem

2.3.1. Sample preparation

Plasma concentrations of diltiazem were determined using a slight modified of the reported HPLC assay Goebel *et al*. (1985). Briefly, 50 μl of imipramine (2 μg/ml; internal standard), and 1.2 ml of tert-butylmethylether were added to a 0.2 ml of samples. The mixture was then stirred for 2 min and centrifuged for (13,000 rpm, 10 min). One ml of the organic layer was transferred to a clean test tube and 0.2 ml of 0.01 N hydrochloride was added and mixed for 2 min. 50 μl of the water layer was injected into the HPLC system.

2.3.2. HPLC condition

The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu, Japan), a UV-Vis detector (Model SPD-10A), a system controller (Model SCL-10A), a degasser (Model DGU-12A) and an autoinjector (SIL-10AD). The mobile phase was methanol : acetonitrile : 0.04 M ammonium bromide : triethylamine (24 : 31 : 45 : 0.1, v/v/v, pH 7.4, adjusted with acetic acid) was run to flow rate of 1.5 ml/min, and the column [μ-bondapack C_{18} column (3.9 mm, i.d. × 300 mm, 10 μm) Waters Co., Ireland] eluent was monitored at 237 nm at room temperature. The retention times of desacetyldiltiazem, diltiazem and internal

standard was 6.9-min, 8.7-min and 9.7-min, respectively (Figure 3). The calibration curves of diltiazem and desacetyldiltiazem were linear within the ranges of 5–500 ng/ml (Figures 4 and Figures 5). The intra- and inter-day (n = 5) coefficients of variation were less than 5% for diltiazem and desacetyldiltiazem, respectively. Detection limit of diltiazem and desacetyldiltiazem was 10 ng/ml

2.4. Pharmacokinetic analysis

The following pharmacokinetic data were analyzed using the non-compartmental method (WinNonlin software version 4.1; Pharsight Corporation, Mountain View, CA, USA). The half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and the time to reach peak concentration (t_{max}) of diltiazem or desacetyldiltiazem directly read from the experimental data.. The area under the plasma concentration time-curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area was determined by C_{last}/K_{el}. The relative bioavailability (RB) was estimated by $AUC_{coadmin}/AUC_{control} \times 100$. The metabolite-parent ratio (MR) was estimated by $AUC_{desacetyldiltiazem}/AUC_{diltiazem}$

2.5. Statistical analysis

Statistical analysis was conducted using a one-way analysis of viariance (ANOVA) followed by *a posteriori* testing with Dunnett correction using the means for the unpaired data. Differences were deemed be significant at a level of $p < 0.05$. All data were expressed in terms of the mean ± S.D.

Table 5. Mean (± S.D.) plasma concentrations of diltiazem after oral administration of diltiazem (15 mg/kg) without or with metformin to rats (n = 6, each).

Time (h)	Control (Diltiazem)	Diltiazem + Metformin	
		10 mg/kg	30 mg/kg
0	0	0	0
0.1	138 ± 33.1	191 ± 45.8	203 ± 48.7
0.25	162 ± 38.8	230 ± 55.2	246 ± 59.0
0.5	112 ± 26.8	148 ± 35.5	158 ± 37.9
1	67 ± 16.1	80.0 ± 19.2	87.0 ± 20.9
2	29.5 ± 7.07	40.0 ± 9.6	43.7 ± 10.5
3	16.7 ± 4.01	25.2 ± 6.05	27.8 ± 6.69
4	13.6 ± 3.24	18.2 ± 4.37	19.8 ± 4.95
8	7.0 ± 1.67	10.3 ± 2.47	11.4 ± 2.85
12	4.2 ± 1.0	6.0 ± 1.44	6.7 ± 1.7
24	3.1 ± 0.73	4.5 ± 1.1	5.2 ± 1.3

Table 6. Mean (± S.D.) plasma concentrations of desacetyldiltiazem after oral administration of diltiazem (15 mg/kg) without or with metformin to rats (*n* = 6, each).

Time (hour)	Control	Diltiazem + Metformin	
		10 mg/kg	30 mg/kg
0	0	0	0
0.1	55.6 ± 13.4	57.0 ± 13.7	61.0 ± 14.6
0.25	64.4 ± 15.3	68.4 ± 16.4	74.5 ± 17.8
0.5	66.1 ± 15.7	71.5 ± 17.2	76.8 ± 18.5
1	48.7 ± 11.6	50.4 ± 12.1	54.6 ± 13.1
2	26.0 ± 6.24	28.5 ± 6.84	31.0 ± 7.44
3	15.7 ± 3.76	19.0 ± 4.56	20.9 ± 5.02
4	11.3 ± 2.71	13.6 ± 3.26	15.0 ± 3.60
8	6.7 ± 1.61	8.9 ± 2.1	10.0 ± 2.51
12	4.6 ± 1.1	6.3 ± 1.6	7.1 ± 1.8
24	3.1 ± 0.75	4.3 ± 1.1	5.1 ± 1.3

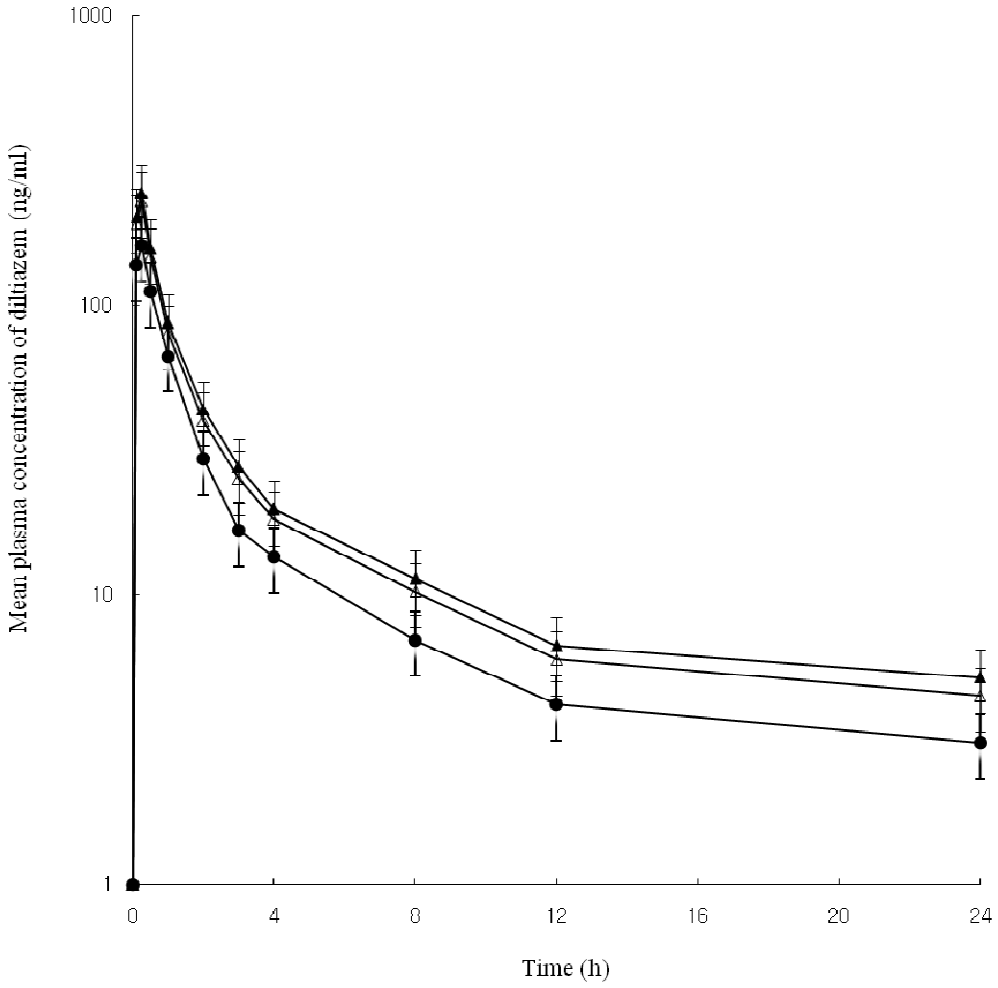

Figure 9. Mean arterial plasma concentration–time profiles of diltiazem after oral administration of diltiazem (15 mg/kg) without (●) or with 10 mg/kg (△) or 30 mg/kg (▲) of metformin to rats (*n* = 6, each). Bars represent the standard deviation.

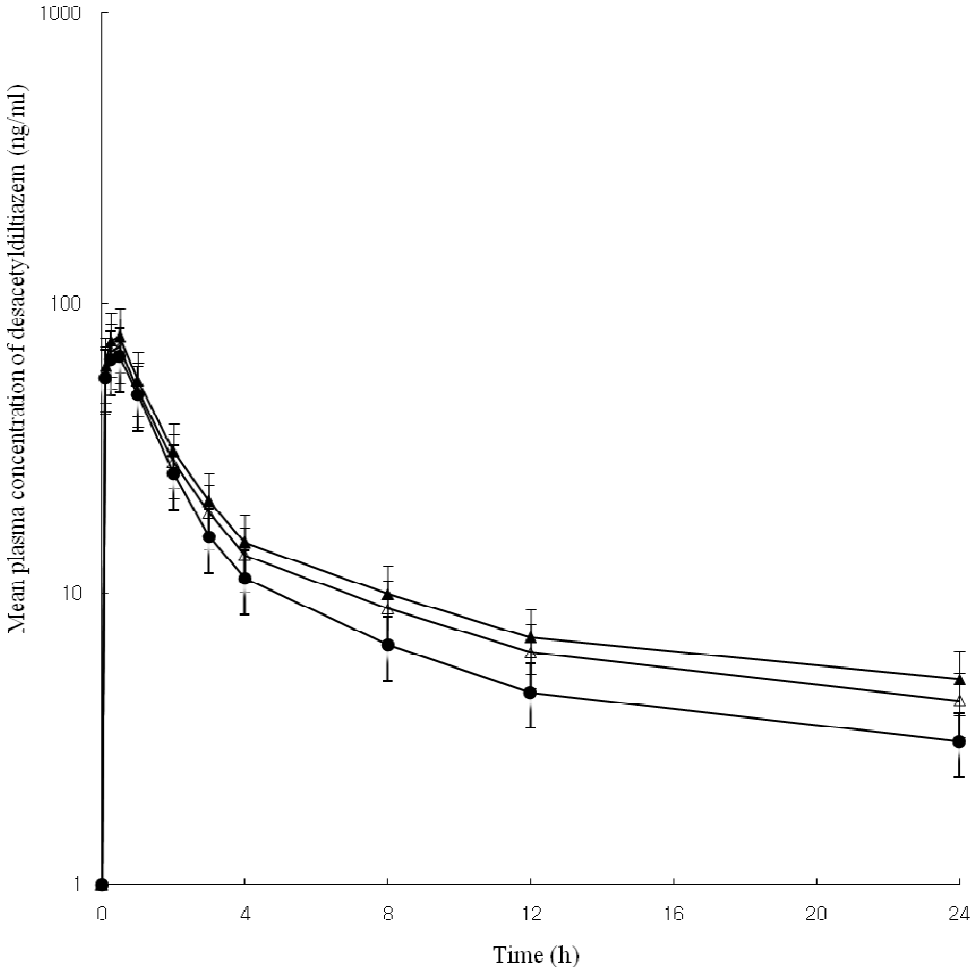

Figure 10. Mean arterial plasma concentration–time profiles of desacetyldiltiazem after oral administration of diltiazem (15 mg/kg) without (●) or with 10 mg/kg (△) or 30 mg/kg (▲) of metformin to rats (n = 6, each). Bars represent the standard deviation.

Table 7. Mean (± S.D.) Pharmacokinetic parameters of diltiazem after oral administration of diltiazem (15 mg/kg) without or with metformin to rats (*n* = 6, each).

Parameter	Control (Diltiazem)	Diltiazem + Metformin	
		10 mg/kg	30 mg/kg
AUC (ng·h/ml)	348 ± 83.5	481 ± 115*	534 ± 128*
C_{max} (ng/ml)	162 ± 38.9	230 ± 55.2*	246 ± 59.0*
T_{max} (h)	0.25	0.25	0.25
$t_{1/2}$ (h)	10.3 ± 2.47	10.7 ± 2.57	11.2 ± 2.69
RB (%)	100	134	154

* $P < 0.05$ compared to control.

AUC: area under the plasma concentration–time curve from 0 h to infinity;

C_{max}: peak plasma concentration;

T_{max}: time to reach peak concentration;

$t_{1/2}$: terminal half-life;

RB: relative bioavailability.

Table 8. Mean (± S.D.) Pharmacokinetic parameters of desacetyldiltiazem after oral administration of diltiazem (15 mg/kg) without or with metformin to rats (*n* = 6, each).

Parameter	Control	Diltiazem + Metformin	
		10 mg/kg	30 mg/kg
AUC (ng·h/ml)	285 ± 68.4	358 ± 85.9	405 ± 98.4*
C_{max} (ng/ml)	66.1 ± 15.8	71.3 ± 17.1	76.8 ± 18.3
T_{max} (h)	0.5	0.5	0.5
$t_{1/2}$ (h)	11.6 ± 2.78	12.8 ± 3.07	13.5 ± 3.28
RB	100	126	141
MR	0.82 ± 0.18	0.74 ± 0.17	0.77 ± 0.17

* $P < 0.05$ compared to control.

$AUC_{0\sim\infty}$: area under the plasma concentration–time curve from 0 h to infinity;

C_{max}: peak plasma concentration;

T_{max}: time to reach peak concentration;

$t_{1/2}$: terminal half-life;

RB: relative bioavailability;

MR: metabolite-parent ratio.

3. Results and discussion

Figure 3 shows the HPLC chromatograms of the rat's blank plasma (A) and the plasma spiked with diltiazem, desacetyldiltiazem and imipramine (internal standard) (B). The retention of diltiazem, desacetyldiltiazem and imipramine was 8.7, 6.7 and 9.7 min, respectively.

The calibration curves of diltiazem (Figure 4) and desacetyldiltiazem (Figure 5) were linear within the concentration ranges from 5–500 ng/ml, respectively. The detection limits for diltiazem and descetyldiltiazem was 5 ng/ml. The intra- and inter-day (n = 5) coefficients of variation were less than 5% for diltiazem and desacetyldiltiazem.

The mean plasma concentration–time profiles of diltiazem after oral administration (15 mg/kg) with or without of metformin (10 or 30 mg/kg) were illustrated in Figure 9, and Table 7 listed the relevant pharmacokinetic parameters of diltiazem after oral administration. Compared with the control group, presence of metformin significantly (P < 0.05) increased the area under the plasma concentration–time curve (AUC) and the peak concentration (C_{max}) of diltiazem. Consequently, the relative bioavailability (RB) of diltiazem increased by 1.34- to 1.54-fold than those of the control group. CYP3A4, a key enzyme for the metabolism of diltiazem is mainly located in liver, and it is also expressed in small intestine (Pichard *et al*., 1990; Watkins *et al*., 1987; Kolars *et al*., 1992). This result appeared to be consistent with previous studies, a single oral dose (fluvastatin, atorvastatin, naringin and morin) significantly increased the AUC and C_{max} of diltiazem in rats, which was due to inhibition of CYP3A4. Metformin increased the terminal half-life ($t_{1/2}$) of diltiazem but not significantly. Metformin did not affect the T_{max} of diltiazem.

The mean plasma concentration–time profiles of desacetyldiltiazem after oral administration of diltiazem (15 mg/kg) with or without metformin (10 or 30 mg/kg)

were shown in Figure 10. As listed in Table 8, 30 mg/kg of metformin significantly (P <0.01) increased the area under the plasma concentration–time curve (AUC) of desacetyldiltiazem. Metformin increased the peak plasma concentration (C_{max}) and the terminal half-life ($t_{1/2}$) of desacetyldiltiazem but not significantly. Consequently, the relative bioavailability (RB) of desacetyldiltiazem increased by 1.35- to 1.51-fold than those of the control group. Compare to the control group, presence of metformin (10 or 30 mg/kg) decreased the metabolite-parent ratio (MR) but not significantly. These results suggest the metabolism and/or absorption of diltiazem may be inhibited by metformin. Metformin did not affect the T_{max} of desacetyldiltiazem.

CYPs in enterocytes contribute significantly to the “first-pass” metabolism and oral bioavailability of many drugs and chemicals. The “first pass” metabolism of compounds in the intestine limits absorption of toxic xenobiotics and may ameliorate adverse effects. Moreover, induction or inhibition of intestinal CYPs may be responsible for significant drug/drug interactions when one agent decreases or increases the F and K_a of a concurrently administered drug (Kaminsky and Fasco, 1991).

The increased bioavailability of diltiazem by metformin suggests that CYP3A could be competitively inhibited by metformin, which resulted in reducing first-pass metabolism of diltiazem in the intestine and/or liver. The adjustment of the dose of diltiazem should be taken into consideration for potential interaction between metformin and diltiazem in clinical setting.

4. Conclusion

Presence of metformin significantly enhanced the systemic bioavailability of diltiazem in rats. If the results are further confirmed in the clinical trial, dose adjustment of diltiazem shoud be taken into consideration when diltiazem is treated with concomitantly with metformin to the patients.

References

Buckley MMT, Grant SM, Goa KL, McTabish D, Sorkin EM. Diltiazem: A reappraisal of its pharmacological properties and therapeutic use. Drugs. 1990; 39: 757–806.

Chaffman M, Brogden RN. Diltiazem: a review of its pharmacological properties and therapeutic efficacy. Drugs. 1985; 29: 387–454.

Choi HJ, Choi JS. Effects of morin pretreatment on the pharmacokinetics of diltiazem and its major metabolite, desacetyldiltiazem in rats. Arch Pharm Res. 2005; 28: 970–976.

Choi JS, Han HK. Pharmacokinetic interaction between diltiazem and morin, a flavonoid, in rats. Pharmacol Res. 2005; 52: 386–391.

Choi JS, Li X. Enhanced diltiazem bioavailability after oral administration of diltiazem with quercetin to rabbits. Int J Pharm. 2005; 297: 1–8.

Choi JS, Piao YJ, Han HK.Pharmacokinetic interaction between fluvastatin and diltiazem in rats. Biopharm Drug Dispos. 2006; 27: 437–441.

Choi YH, Lee MG. Effects of enzyme inducers and inhibitors on the pharmacokinetics of metformin in rats: involvement of CYP2C11, 2D1, and 3A1/2 for the metabolism of metformin. Br J Pharmacol. 2006; 149: 424–430.

Formoso G, De Filippis EA, Michetti N, Di Fulvio P, Pandolfi A, Bucciarelli T, Ciabattoni G, Nicolucci A, Davì G, Consoli A. Decreased *in vivo* oxidative stress and decreased platelet activation following metformin treatment in newly diagnosed type 2 diabetic subjects. Diabetes Metab Res Rev. 2008; 24: 231–237.

Gan LSL, Moseley MA, Khosla B, Augustijns PF, Bradshaw TP, Hendren RW, Thakker DR. CYP3A-Like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells: interaction between the two biochemical barriers to intestinal transport. Drug Metab Dispos. 1996; 24: 344–

349.

Goebel KJ, Kolle EU. High performance liquid chromatographic dertermination of diltiazem and four of its metabolites in plasma. J Chromatogr. 1985; 345: 355–363.

Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem. 1993; 62: 385–427.

Homsy W, Caille G, du Souich P. The site of absorption in the small intestine determines diltiazem bioavailability in the rabbit. Pharm Res. 1995a; 12: 1722–1726.

Homsy W, Lefebvre M, Caille G, du Souich P. Metabolism of diltiazem in hepatic and extrahepatic tissues of rabbits: *in vitro* studies. Pharm Res. 1995b; 12: 609–614.

Hong SP, Chang KS, Choi DH, Choi JS. Effect of atorvastatin on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats. Arch Pharm Res. 2007; 30: 90–95.

Ito K, Kusuhara H, Sugiyama Y. Effects of intestinal CYP3A4 and P-glycoprotein on oral drug absorption theoretical approach. Pharm Res. 1999; 16: 225–231.

Iwao T, Inoue K, Hayashi Y, Yuasa H, Watanabe J. Absorption and metabolic extraction of diltiazem from the perfused rat small intestine, Drug Metab Pharm. 2004; 19: 430–437.

Kaminsky LS, Fasco MJ. Small intestinal cytochromes P450. Crit Rev Toxicol 1991; 21: 407–422.

Kolars JC, Schmiedlin-Ren P, Dobbins WO, Schuetz J, Wrighton SA, Watkins PB. Heterogeneity of cytochrome P450IIIA expression in rat gut epithelia. Gastroenterology. 1992; 102: 1186–1198.

LeBoeuf E, Grech-Belanger O. Deacetylation of diltiazem by rat liver. Drug Metab Dispos. 1987; 15: 122–126.

Lee YH, Lee MH, Shim CK. Pharmacokinetics of diltiazem and deacetyldiltiazem

in rats. Int J Pharm. 1991; 76: 71–76.

Lefebvre M, Homsy W, Caille G, du Souich P. First-pass metabolism of diltiazem in anesthetized rabbits: role of extrahepatic organs. Pharm Res. 1996; 13: 124–128.

Mukherjee R, Jow L, Croston GE, Paterniti JR. Identification, characterization, and tissue distribution of human peroxisome proliferator−activated receptor (PPAR) isoforms PPARγ2 versus PPARγ1 and activation with retinoid X receptor agonists and antagonists. J Biol Chem. 1997; 272: 8071–8076.

Narita H, Otsuka M, Yabana H, Nagao T. Hypotensive response of spontaneously hypertensive rats to centrally administered diltiazem and its metabolites: in relevance to the hypotensive action by oral administration. J Pharmacobiodyn. 1986; 9: 547–553.

Pichard L, Gillet G, Fabre I, Dalet-Beluche I, Bonfils C, Thenot JP, Maurel P. Identification of the rabbit and human cytochromes P-450IIIA as the major enzymes involved in the N-demethylation of diltiazem. Drug Metab Dispos. 1990; 18: 711–719.

Robert F, Fendri S, Hary L, Lacroix C, Andréjak M, Lalau JD. Kinetics of plasma and erythrocyte metformin after acute administration in healthy subjectsDiabetes Metab. 2003; 29: 279–283.

Sahi J, Black CB, Hamilton GA, Zheng X, Jolley S, Rose KA, Gilbert D, LeCluyse EL, Sinz MW. Comparative effects of thiazolidinediones on *in vitro* P450 enzyme induction and inhibition. Drug Metab Dispos. 2003; 31: 439–446.

Scheen AJ. Pharmacokinetic interactions with thiazolidinediones. Clin Pharmacokinet. 2007; 46: 1–12.

Schiffrin EL, Amiri F, Benkirane K, Iglarz M, Diep QN. Peroxisome proliferator−activated receptors: vascular and cardiac effects in hypertension. Hypertension. 2003; 42: 664–668.

Setter SM, Iltz JL, Thams J, Campbell RK. Metformin Hydrochloride in the Treatment of Type 2 Diabetes Mellitus: A Clinical Review with a Focus on

Dual Therapy Campbell. Clin Ther 2003; 25: 2991–3026.

Wacher VJ, Silverman JA, Zhang Y, Benet LZ. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. J Pharm Sci. 1998; 87: 1322–1330.

Wacher VJ, Salphati L, Benet LZ. Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv Drug Deliv Rev. 2001; 46: 89–102.

Walsky RL, Gaman EA, Obach RS. Examination of 209 drugs for inhibition of cytochrome P450 2C8. J Clin Pharmacol. 2005; 45: 68–78.

Watkins PB, Wrighton SA, Schuetz EG, Molowa DT, Guzelian PS. Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man. J Clin Invest. 1987; 80: 1029–1036.

Weir MR. Diltiazem: ten years of clinical experience in the treatment of hypertension. J Clin Pharmacol. 1995; 35: 220–232.

Yeung PK, Prescott C, Haddad C, Montague TJ, McGregor C, Quilliam MA, Xei M, Li R, Farmer P, Klassen GA. Pharmacokinetics and metabolism of diltiazem in healthy males and females following a single oral dose. Eur J Drug Metab Pharmacokinet. 1993; 18: 199–206.

Yeung PK, Feng JDZ, Buckley SJ. Pharmacokinetics and hypotensive effect of diltiazem in rabbits: Comparison of diltiazem with its major metabolites. J Pharm Pharmacol. 1998; 50: 1247–1253.

Yusa K, Tsuruo T. Reversal mechanism of multidrug resistance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. Cancer Res. 1989; 49: 5002–5006.

Acknowledgements

약간 선선한가 했더니 어느덧 무더운 여름으로 달려가고 있는 계절이 다가오며 어렵게만 보였던 박사과정을 마치는 시기가 되었습니다. 시간은 주마등같이 흐른다는 것이 새삼 느껴집니다.

새로운 배움과 학위를 취득하며 무언가를 이루었다는 성취감에 마음이 뿌듯하며 이 모든 것을 이루는데 밑거름이 되게하신 분들께 감사의 말을 전하고자 합니다.

좋은 연구 테마의 조언으로 학위의 꿈을 주신 최준식 교수님께 머리숙여 감사드리오며 연구 시 옆에서 도와주신 박영길 선생님께도 감사드리며, 부모님과 집사람에게도 한없는 감사를 드립니다.

학위를 가지고 세상에 밑거름이 되며 그리고, 더욱 더 매진하는 모습으로 한걸음 한걸음 나아가겠습니다. 그리고, 세상의 밀알과 촛불이 되는 사람이 되겠습니다.

마지막으로, 날마다 숨쉬는 순간마다 저를 지키시는 하나님께 감사드립니다.

저작물 이용 허락서

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논문제목	한글: 흰쥐에서 피오글리타존 또는 메트폴민과 딜티아젬의 약물동태학적 상호작용. 영문: Drug interaction between pioglitazone or metformin and diltiazem in rats				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함.
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의 (√) 반대 ( )

2008 년 02 월

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