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# The Influence of Cimetidine on the Pharmacokinetics of Diltiazem and its Main Metabolite in Rabbits

**Jun Shik Choi and Jin Pil Burm[1]**

*College of Pharmacy, Chosun University, Kwangju 501-759, Korea and [1]Chosun Nursing College, Kwangju 501-140, Korea*

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The purpose of this study was to investigate the pharmacokinetic alteration of diltiazem and its main metabolite, deacetyldiltiazem, after oral administration of diltiazem in rabbits with or without cimetidine co-administration. The area under the plasma concentration-time curve (AUC) of diltiazem was significantly elevated in rabbits pretreated with cimetidine, suggesting that the oral clearance, an index of intrinsic clearance, may be decreased by the cimetidine treatment. Consistent with the increased AUC by the treatment, peak plasma concentration ($C_{max}$) for diltiazem was also elevated. Apparent volume of distribution normalized by the bioavailability ($V_d$/F) of diltiazem increased significantly in rabbits pretreated with cimetidine increased. Taken together with the fact that the first pass metabolism for diltiazem is the primary determinant for the oral bioavailability, these observations indicate that increases in the oral clearance and $V_d$/F may be a manifestation of the decreased first pass metabolism. Consistent with the hypothesis, the AUC of deacetyldiltiazem was significantly decreased in rabbits with cimetidine treatment. Ratio of deacetyldiltiazem to total diltiazem in the plasma was significantly decreased in rabbits with cimetidine treatment. These observations suggested that the metabolism of diltiazem to deacetyldiltiazem was reduced by cimetidine treatment and that the dosage of diltiazem should be adjusted when the drug is co-administered chronically with cimetidine in a clinical setting.

**Key words:** Pharmacokinetics, Diltiazem, Deacetyldiltiazem, Cimetidine

## INTRODUCTION

Diltiazem inhibits voltage-dependent L-type calcium channels, which leads to relaxation of vascular smooth muscle and, ultimately, to negative inotroic and chronotropic effects in the heart (Scholz, 1997). Diltiazem is reported to dilate renal vasculature and can increase the glomerular filtration rate and renal sodium excretion (Epstein and Loutzenhiser, 1990; Ruilope and Alcazar, 1990; Sterzel, 1987). The pharmacokinetics of diltiazem has been studied extensively in the literature. Diltiazem has a large volume of distribution because of its liphophilicity and is rapidly and extensively distributed into body tissues (Hermann *et al.*, 1983). Although the drug readily penetrates the intestinal epithelium, the oral bioavailability is not complete because of a considerable first-pass hepatic metabolism (Bianchetti *et al.*, 1991; Eichelbaum and Echizen, 1984). Consistent with the considerable first pass effect, diltiazem is rapidly and almost completely metabolized in the liver *via* deacetylation (Homsy *et al.*, 1995; Leboeuf *et al.*, 1987), *N*-demethylation and *O*-demethylation. In the literature, a number of metabolites have been identified as a result of the metabolism *via* cytochrome P-450 enzyme system (Murray and Butler, 1996; Sutton *et al.*, 1994). In addition, phase II metabolism for the drug and its metabolites may lead to formations of glucuronide and sulfate forms (Sakuma *et al.*, 1971). We previously reported that the pharmacokinetic changes of diltiazem and its main metabolite, deacetyldiltiazem after oral and intravenous administration of diltiazem to normal rabbits and folate-induced renal failure rabbits (Choi and Burm, 2001; Choi and Burm, 2000). Cimetidine is widely prescribed histamine $H_2$ antagonist. The histamine $H_2$ antagonist is a well-known inhibitor of cytochrome P-450 monooxygenases (Knodell *et al.*, 1991) and the interaction has been reported to have clinical significance. For example, pharmacokinetic interactions

Correspondence to: Jin Pil Burm, Chosun Nursing College, 280, Seosuk-Dong, Dong-Gu, Kwangju 501-140, Korea
Tel: 82-62-231-7361, Fax: 82-62-222-5414
E-mail: jpburm@venus.cnc.ac.kr

between cimetidine and other calcium channel inhibitors, verapamil (Johnson *et al.*, 1995; Mikus *et al.*, 1990; Smith *et al.*, 1984) and nifedipine (Khan *et al.*, 1991; Schwartz *et al.*, 1998; Kirch *et al.*, 1984) have been reported. But interactions between cimetidine and diltiazem have not reported in rabbits.

The purpose of this study was to investigate the pharmacokinetic changes of diltiazem and its main metabolite, deacetyldiltiazem, after oral administration of diltiazem in rabbits with cimetidine treatment. Our data indicate that the cimetidine co-administration may lead to a reduction in the first pass metabolism of diltiazem, thereby elevation of plasma concentration of the drug.

## MATERIALS AND METHODS

### Materials

Diltiazem and deacetyldiltiazem were kindly supplied Hanil Pharm. Co. (Seoul, Korea). Imipramine, *tert*-butyl methyl ether, ammonium bromide and triethylamine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals were reagent grade and were used without further purification. HPLC (Model CBM-10A, Shimadzu Co., Kyoto, Japan), syringe pump (Model 341B, Sage Co., Kyoto, Japan), vortex mixer (Scientific Industries, Seoul, Korea) and centrifuger (Abbot Co., TM, USA) were also used in this study.

### Animals

White male New Zealand rabbits weighing 2.0~2.5 kg were fasted at least 24 h prior to the experiment and were given water freely. Under 25% urethane (4 mL/kg) anesthesia, the right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, USA) for blood sampling. After a pre-determined recovery period, drug administration was initiated.

### Drug administration and Blood sampling

Diltiazem (dose: 20 mg/kg) was orally administered to rabbits with or without cimetidine treatment. Cimetidine treatment consisted of the four consecutive daily oral administration of cimetidine dose of 20 mg/kg and subsequent co-administration of diltiazem and cimetidine (20 mg/kg) orally at the fifth day. Blood samples (1.5 mL) were withdrawn from the femoral artery at 7.5, 15, 30 min, 1, 2, 4, 6, 9, 12, and 24 h after the diltiazem administration. Blood sample was centrifuged at 3,000 rpm for 10 min and an aliquot (0.5 mL) of the supernatants collected. Plasma samples were then stored at -70°C until the analysis. Throughout the pharmacokinetic study, saline was infused at the rate of 1.5 mL/h to ear vein by an infusion pump.

### Analysis of diltiazem and deacetyldiltiazem in rabbit plasma

Plasma concentration of diltiazem and deacetyldiltiazem was determined by a HPLC-UV assay essentially described by Goebel and Kolle (1985). Briefly, an aliquot (0.2 mL) of a solution containing imipramine (internal standard, 0.25%) and *tert*-butyl methyl ether (5 mL) were added to 0.5 mL of plasma sample. The mixture was then vortexed for 5 min and centrifuged at 6,000 rpm for 5 min. An aliquot of the organic layer (4.5 mL) was transferred to a clean test tube containing 0.3 mL of 0.01 N hydrochloric acid. The mixture was then vortexed for 5 min and centrifuged at 6,000 rpm for 5 min. The upper layer was discarded and 50 μL of the aqueous layer was injected onto the HPLC system.

The HPLC separation was carried out at room temperature detector and the wavelength set at 254 nm. The stationary phase was a Shin-pack CLC-ODS column (4.6 ×250 mm, Shimadzu Co., Kyoto, Japan). Mixtures of methanol : acetonitrile : 0.04 M ammonium bromide : triethylamine (24:31:45:0.1, v/v/v/v, pH 6.43) were used as the mobile phase in this study. The mobile phase was filtered by passing through a 0.45 μm pore size membrane filter. At a flow rate of 1.5 mL/min, the retention times for deacetyldiltiazem, the internal standard and diltizem were 5.3 min, 8.2 min, and 10.5 min, respectively.

### Pharmacokinetic analysis

Pharmacokinetic parameters were calculated with a nonlinear least square regression using a MULTI program (Yamaoka *et al.*, 1971). The parameter value was obtained by the use of simplex algorithm. The area under the plasma concentration-time curves (AUC) from time zero to time infinity was calculated by trapezoidal rule up to the last collection time and the standard area extrapolation method. Oral clearance [i.e., total body clearance ($CL_t$) normalized by the bioavailability] was calculated by the division of the dose to AUC. The apparent volume of distribution was calculated by the following equation.

$$V_d = F \cdot \text{Dose} / K_{el} \cdot \text{AUC}.$$

The F value was calculated by the division of AUC from time zero to infinity after the oral administration by the AUC from time zero to infinity after the intravenous administration of diltiazem at the identical dose. The intravenous AUC data was taken from our previous report (Choi and Burm, 2000). The maximum plasma concentration ($C_{max}$) and time to reach the maximum plasma concentration ($T_{max}$) were obtained directly from plasma concentration-time curves.

When it was necessary, metabolite percentage rate (%) of deacetyldiltiazem in rabbits was calculated;

$$\text{Metabolite percentage rate} = \frac{\text{Plasma concentration of diltiazem plus deacetyldiltiazem}}{\text{Plasma concentration of deacetydiltiazem}} \times 100$$

The rate was then compared for control and cimetidine pretreatment.

### Statistical analysis

All means were presented with their standard deviation (Mean ± S.D.). The two way ANOVA was utilized to determine a significance difference between control rabbits and rabbits pretreated with cimetidine. Differences were considered to be significant at $p<0.05$.

## RESULTS AND DISCUSSION

### Plasma concentrations of diltiazem and deacetyldiltiazem

The plasma concentration of diltiazem and deacetyldiltiazem after oral administered diltiazem in rabbits coadministered and pretreated with cimetidine were showed in Fig. 1 and Fig. 2, respectively. The plasma concentration of diltiazem in rabbits pretreated with cimetidine increased significantly ($p<0.05$) from 9 h to 24 h compared with those of the control rabbits. The plasma concentration of deacetyldiltiazem in rabbits pretreated with cimetidine decreased significantly ($p<0.05$) from 9 h to 24 h for corresponding concentrations of the control rabbits.

### Pharmacokinetic parameters of diltiazem

The pharmacokinetic parameters of diltiazem in control rabbits and in rabbits coadministered and pretreated with cimetidine were shown in Table I. The $C_{max}$ and $T_{max}$ of diltiazem in rabbits pretreated with cimetidine increased

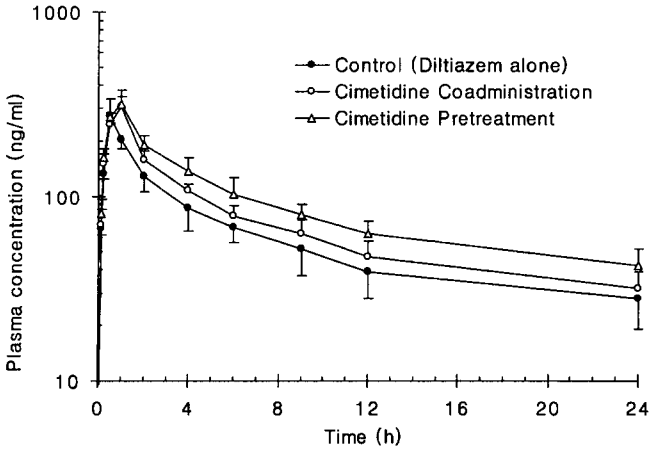


**Fig. 1.** Plasma concentration - time profiles of diltiazem after oral administration (20 mg/kg) in rabbits pretreated with cimetidine. Bars represent Mean ± S.D. (n = 6).

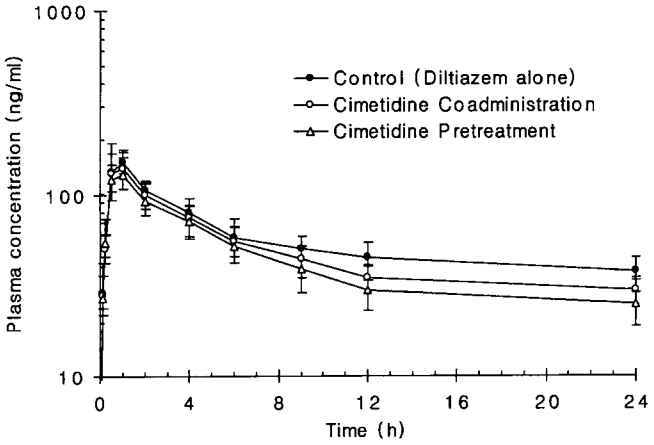


**Fig. 2.** Plasma concentration - time profiles of deacetyldiltiazem after oral administration (20 mg/kg) in rabbits pretreated with cimetidine. Bars represent Mean ± S.D. (n = 6).

Table I. Pharmacokinetic parameters of diltiazem after oral administration (20 mg/kg) in rabbits coadministered or pretreated with cimetidine

| Parameters | Control (Diltiazem alone) | Cimetidine | |
|---|---|---|---|
| | | Coadministration | Pretreatment |
| $K_{el}$ ($h^{-1}$) | 0.045± 0.006 | 0.041± 0.008 | 0.038± 0.005 |
| $t_{1/2}$ (h) | 15.3 ± 2.58 | 16.9 ± 3.27 | 18.2 ± 2.58 |
| $C_{max}$ (ng/mL) | 295 ± 38.2 | 312 ± 53.5 | 346 ± 2.24* |
| $T_{max}$ (min) | 37.5 ± 7.45 | 55.0 ± 9.99 | 50.0 ± 6.82* |
| $V_d/F$ (L/kg) | 76.9 ± 10.2 | 84.6 ± 13.9 | 91.6 ± 12.4* |
| $CL_t/F$ (L/h/kg) | 3.46 ± 0.42 | 3.47 ± 0.67 | 3.48 ± 0.54 |
| AUC (ng/mL·h) | 1963 ±347 | 2299 ±525 | 2927 ±317** |
| F[a] | 0.34 ± 0.064 | 0.40 ± 0.096 | 0.51 ± 0.071 |

Mean ± S.D (n=6), *$p<0.05$, **$p<0.01$ compared with control.

[a]F values were calculated by the I.V data (AUC; 863 ng/mL·h, Dose; 3 mg/kg) reported previously. $K_{el}$, elimination rate constant; $t_{1/2}$, terminal half-life; $C_{max}$, peak concentration; $T_{max}$, time of peak concentration; $V_d$, volume of distribution at steady state; $CL_t$, total body clearance; AUC, area under the plasma concentration-time curve from time zero to time infinity.

**Table II.** Pharmacokinetic parameters of deacetyldiltiazem after oral administration (20 mg/kg) in rabbits coadministered or pretreated with cimetidine

| Parameters | Control (Diltiazem alone) | Cimetidine | |
|---|---|---|---|
| | | Coadministration | Pretreatment |
| Terminal phase slope ($h^{-1}$) | 0.023± 0.003 | 0.031± 0.006* | 0.036± 0.006** |
| $t_{1/2}$ (h) | 30.4 ± 5.49 | 22.3 ± 4.48* | 19.2 ± 2.97** |
| $C_{max}$ (ng/mL) | 174 ± 24.8 | 158 ± 29.2 | 142 ± 39.4 |
| $T_{max}$ (min) | 66.0 ± 8.24 | 60.0 ± 7.11 | 55.0 ± 4.28 |
| AUC (ng/mL·h) | 3012 ±428 | 2041 ±329** | 1662 ±214** |

Mean ± S.D (n=6), *p<0.05, **p<0.01 compared with control. $K_{el}$, elimination rate constant; $t_{1/2}$, terminal half-life; $C_{max}$, peak concentration; $T_{max}$, time of peak concentration; AUC, area under the plasma concentration-time curve from time zero to time infinity.

and prolonged significantly ($p<0.05$) to the control rabbits. The $V_d$ of diltiazem in rabbits pretreated with cimetidine (91.6 ± 12.4 L/kg) increased significantly ($p < 0.05$) to the control rabbits (76.9 ± 10.2 L/kg). The AUC of diltiazem in rabbits pretreated with cimetidine (2927 ± 317 ng/mL·h) increased significantly ($p<0.01$) to the control rabbits (1963 ± 347 ng/mL·h).

## Pharmacokinetic parameters of deacetyldiltiazem

The pharmacokinetic parameters of deacetyldiltiazem in control rabbits and in rabbits coadministered and pretreated with cimetidine were shown in Table II. The terminal phase slope of deacetyldiltiazem in rabbits pretreated with cimetidine increased significantly ($p<0.05$) compared with that of control rabbits. As expected, the half life of the terminal phase decreased ($p<0.01$). The AUC of deacetyldiltiazem in rabbits pretreated with cimetidine (2041 ± 329 ng/mL·h and 1662 ± 214 ng/mL·h) decreased significantly ($p < 0.01$) compared with control rabbits (3012 ± 428 ng/mL·h).

## Metabolite percentage rate of deacetyldiltiazem

Metabolite percentage rate of deacetyldiltiazem in rabbits pretreated with cimetidine was shown in Fig. 3. The metabolite percentage rate of deacetyldiltiazem in rabbits coadministered and pretreated with cimetidine inhibited significantly after 12 h and 1 h, respectively, to the control rabbits. The mean metabolite percentage rate of deacetyldiltiazem in rabbits pretreated with cimetidine (31.4 ± 4.09 %) inhibited significantly ($p<0.01$) to the control rabbits (43.4 ± 6.79%).

In this study, the $V_d/F$ of diltiazem in rabbits pretreated with cimetidine increased significantly compared with the control rabbits. In addition, the oral AUC of diltiazem in rabbits pretreated with cimetidine increased by 149% significantly to the control rabbits. In the literature, it has been indicated that the drug readily penetrates the intestinal epithelium while the oral bioavailability is not complete because of a considerable first-pass hepatic metabolism (Bianchetti *et al.*, 1991; Eichelbaum and

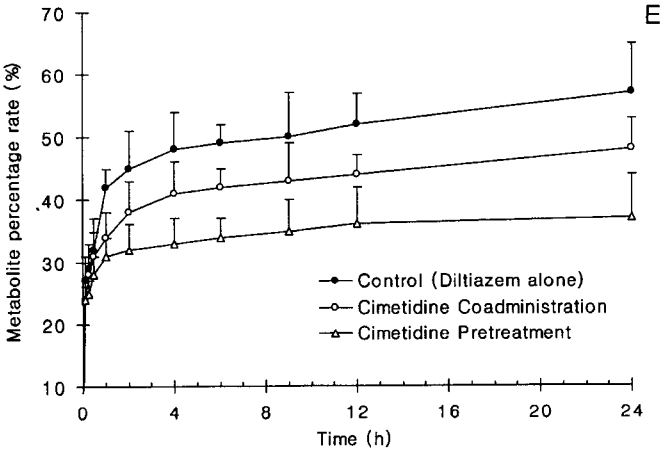


**Fig. 3.** Metabolite percentage rate of deacetyldiltiazem to the plasma concentration of total diltiazem in rabbits pretreated with cimetidine. Bars represent Mean ± S.D. (n = 6). Metabolite percentage rate = Plasma concentration of deacetyldiltiazem/Plasma concentration of diltiazem plus deacetyldiltiazem × 100.

chizen, 1984). Taken together with the fact that cimetidine is a known inhibitor for CYP450 monooxygenases (Knodell *et al.*, 1991), the change in diltiazem pharmacokinetics after the oral administration may be a manifestation of reduced first pass metabolism (i.e., increase in F). Consistent with the hypothesis, the AUC of deacetyldiltiazem in rabbits coadministered and pretreated with cimetidine decreased by 67.8% and 55.2% significantly to the control rabbits. However, in a report by Winship *et al.* (1985), the authors reported that the deacetyldiltiazem plasma concentration increased during concurrent cimetidine administration. The underlying mechanism(s) for the discrepancy has not been directly investigated in this study. However, differences in experimental design may have been contributed for the dissociation in diltiazem pharmacokinetics.

Johnson *et al.* (1995) reported that cimetidine coadministration had no significant effect on the apparent volume of distribution of verapamil, other calcium channel inhibitor. On the other hand, the oral clearance of verapamil administered was reduced by 28% during cimetidine coadminis-

tration. Consistent with the observation, Mikus *et al.* (1990) reported that the increase in plasma concentration of verapamil produces a more pronounced pharmacological effect of verapamil when cimetidine is coadministered. Also, cimetidine had no effect on intravenous verapamil pharmacokinetics but induced a significant rise in oral verapamil bioavailability (Smith *et al.*, 1984). In nifedipine, other calcium channel inhibitor, cimetidine produced significant changes in the AUC of nifedipine at steady state (Khan *et al.*, 1991; Kirch *et al.*, 1984) and after single doses of nifedipine, cimetidine decreased apparent oral clearance (Schwartz *et al.*, 1998). Therefore, the interaction between the cimetidine and calcium channel blockers is apparently complex, and, thus, clinicians have to be extremely cautious in the selection of the calcium channel blocker when the patients are already taking cimetidine. Therefore, these results suggest that the dosage of diltiazem may have to be adjusted when the drug would be administered chronically with cimetidine in a clinical situation.

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