Effects of Ticlopidine on the Pharmacokinetics of Diltiazem and Its Main Metabolite, Desacetyldiltiazem, in Rats

Jun-Shik Choi¹, Joon-Seung Yang² and Dong-Hyun Choi²*¹

¹College of Pharmacy, ²College of Medicine, Chosun University, Gwangju 501-759, Korea

Abstract
The purpose of this study was to investigate the effect of ticlopidine on the pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem, in rats. Pharmacokinetic parameters of diltiazem and desacetyldiltiazem were determined in rats after oral administration of diltiazem (15 mg · kg⁻¹) with ticlopidine (3 or 9 mg · kg⁻¹). The effects of ticlopidine on P-glycoprotein (P-gp) and cytochrome P450 (CYP) 3A4 activities were also evaluated. Ticlopidine inhibited CYP3A4 enzyme activity in a concentration-dependent manner with a 50% inhibition concentration (IC₅₀) of 35 μM. In addition, ticlopidine did not significantly enhance the cellular accumulation of rhodamine-123 in NCI/ADR-RES cells overexpressing P-gp. Compared with the control (given diltiazem alone), ticlopidine significantly altered the pharmacokinetic parameters of diltiazem. The peak concentration (Cₘ₉₅) and the area under the plasma concentration-time curve (AUC) of diltiazem were significantly (9 mg · kg⁻¹, p<0.05) increased in the presence of ticlopidine. The AUC of diltiazem was increased by 1.44-fold in rats in the presence of ticlopidine (9 mg · kg⁻¹). Consequently, the absolute bioavailability (A.B.) of diltiazem in the presence of ticlopidine (9.3-11.5%) was significantly higher (9 mg · kg⁻¹, p<0.05) than that in the control group (8.0%). Although ticlopidine significantly (p<0.05) increased the AUC of desacetyldiltiazem, the metabolite-parent AUC ratio (M.R.) in the presence of ticlopidine (9 mg · kg⁻¹) was significantly decreased compared to that in the control group, implying that ticlopidine could effectively inhibit the metabolism of diltiazem. In conclusion, the concomitant use of ticlopidine significantly enhanced the oral bioavailability of diltiazem in rats by inhibiting CYP3A4-mediated metabolism in the intestine and/or liver rather than by inhibiting intestinal P-gp activity or renal elimination of diltiazem.

Key Words: Diltiazem, Desacetyldiltiazem, Ticlopidine, Pharmacokinetics, CYP3A4, Rat

INTRODUCTION
Diltiazem is a calcium channel blocker that is widely used for the treatment of angina, supraventricular arrhythmias and hypertension (Chaffman and Brogden, 1985; Yeung et al., 1993; Weir, 1995). Diltiazem undergoes extensive and complex phase I metabolism including desacetylation, N-demethylation, and O-demethylation. The absolute bioavailability of diltiazem is approximately 40%, with large inter-subject variability (Buckley et al., 1990; Yeung et al., 1993). In preclinical studies, the estimated hypotensive potency of desacetyldiltiazem appeared to be about one-half to equivalent to that of diltiazem, whereas the potencies of N-demethyldiltiazem and N-demethyl/N-desacetyldiltiazem were about one-third the potency of diltiazem (Narita et al., 1986; Yeung et al., 1998). Considering the potential contribution of active metabolites to the therapeutic outcome of diltiazem treatment, it may be important to monitor the active metabolites as well as the parent drug in pharmacokinetic studies of diltiazem. CYP3A4, a key enzyme in the metabolism of diltiazem is mainly located in 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 both the small intestine and the 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, indicating that diltiazem is highly extracted in the small intestine and the liver. In addition to the extensive metabolism, P-glycoprotein (P-gp) may also lower the bioavailability of diltiazem. Yusa and Tsuruo (1989) reported that calcium channel blockers such as verapamil and diltiazem competitively restrained the multidrug resistance of P-gp. Wacher et al. (2001) also suggested that diltiazem is a substrate of both CYP3A4 and P-gp. Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically in the presystemic drug...
metabolism, resulting in limited absorption of drugs (Gan et al., 1996; Wacher et al., 1998; Ito et al., 1999; Wacher et al., 2001).

Ticlopidine is extensively metabolized in the liver and is a potent inhibitor of platelet aggregation induced by adenosine diphosphate (ADP), whereas its ability to inhibit aggregation caused by thrombin, collagen, arachidonic acid, adrenaline, and platelet-activating factor varies (Saltiel and Ward, 1987). It has been tried in a variety of platelet-dependent disease states (Gent et al., 1989; Hass et al., 1989; Janzon et al., 1990). Indeed, several recent reviews recommend ticlopidine as a valuable alternative when patients cannot tolerate aspirin (Haynes et al., 1998; Verhaeghe, 1991; Ito et al., 1992; Solomon and Hart, 1994; Buur et al., 1997; Ko et al., 2000).

Ticlopidine is a novel antiplatelet drug reported to cause significant inhibition of several drugs metabolized by the hepatic cytochrome P-450 enzyme system, including antipyrine and theophylline. For example, ticlopidine co-medication results in a significant increase in mean warfarin concentrations (Gidal et al., 1995). There is also report that the oral bioavailability of ticlopidine administered with a meal was increased by 20% and the absorption of ticlopidine administered with antacid was approximately 20% lower than those under fasting conditions (Shah et al., 1990). We therefore evaluated the inhibition of CYP enzyme activity and P-gp activity by ticlopidine using the CYP inhibition assays and rhodamine-123 retention assays in P-gp-overexpressing NCI/ADR-RES cells.

Clinically, diltiazem and ticlopidine can be prescribed for treatment and prevention of cardiovascular disease. However, pharmacokinetic interaction between ticlopidine and diltiazem has not been reported in vivo. Therefore, the present study aims to investigate the effect of ticlopidine on the bioavailability and pharmacokinetics of diltiazem and its active metabolite, desacetyldiltiazem, after oral and intravenous administration of diltiazem in rats.

**MATERIALS AND METHODS**

**Materials**

Diltiazem hydrochloride, desacetyldiltiazem, imipramine hydrochloride and ticlopidine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, methanol, and tert-butylmethyl ether were obtained from Merck Co. (Darmstadt, Germany). All other chemicals were reagent grade and all solvents were HPLC grade.

**Animal studies**

Male Sprague-Dawley rats (270-300 g) were purchased from Dae Han Laboratory Animal Research and Co. (Chooongbuk, Korea) and were given a normal standard chow diet (No. 322-71; Superfeed Co., Gangwon, 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°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 of Chosun University approved the present study.

The rats were divided into six groups (n=6, each): an oral control group (15 mg · kg⁻¹ of diltiazem dissolved in distilled water, 3.0 ml/kg) without or with 3 or 9 mg · kg⁻¹ of ticlopidine (mixed in distilled water, 3.0 ml/kg), and an i.v. group (5 mg · kg⁻¹ of diltiazem, dissolved in 0.9% NaCl solution, 1.5 ml/kg) without or with 3 or 9 mg · kg⁻¹ of oral ticlopidine (mixed in distilled water, 3.0 ml/kg). Sprague-Dawley rats were fasted for at least 24 h prior to the experiment and were given water freely. Each rat was anaesthetized with ether and the right femoral artery was cannulated with polyethylene tubing for blood sampling. Blood was collected from the femoral artery at 0.1, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hr post-dose. Blood samples were centrifuged and the plasma was removed and stored at -80°C until analyzed by HPLC.

**HPLC assay**

Plasma concentrations of diltiazem were determined by an HPLC assay modified from the method of Goebel and Kolle (Goebel and Kolle, 1985). Briefly, 50 μl of imipramine (2 μg · ml⁻¹), as the internal standard, and 1.2 ml of tert-butylmethyl ether were added to 0.2 ml of the plasma samples. The mixture was then stirred for 2 min and centrifuged for 10 min. 1 ml of the organic layer was transferred to a clean test tube and 0.2 ml of 0.01 N hydrochloric acid was added and mixed for 2 min. 50 μl of the water layer was injected into the HPLC system. The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu Co., Japan), a UV-Vis detector (Model SPD-10A), a system controller (Model SCL-10A), degasser (Model DGU-12A) and an autosampler (SIL-10AD). The UV detector was set to 237 nm. The stationary phase was a μ-bondapack C₈ column (3.9×300 mm, 10 μm, Waters Co., Ireland) and the mobile phase was methanol/acetonitrile/0.04 M ammonium bromide/triethylamine (24:31:45:0.1, v/v/v/v, pH 7.4, adjusted with acetic acid). The retention times at a flow rate of 1.5 ml/min were as follows: internal standard at 10.5 min, diltiazem at 8.0 min and desacetyldiltiazem at 6.5 min. The calibration curves of diltiazem and desacetyldiltiazem were linear within the range of 10-400 ng · ml⁻¹. The intra-day (n=5) and inter-day (n=5) coefficients of variation were less than 5% for diltiazem and desacetyldiltiazem, and 1.5% for imipramine. Recovery (%) assessed by replicate analysis (n=5) for five days after adding 20 ng · ml⁻¹ and 200 ng · ml⁻¹ of diltiazem to rat plasma was 106 ± 5.7% and 101 ± 4.9%, respectively. The detection limit of diltiazem and desacetyldiltiazem was 10 ng · ml⁻¹.

**CYP inhibition assay**

The inhibition assays of the human CYP3A4 activities were performed in multiwell plates using the CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously (Crespi et al., 1997). Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrates [50 mM 7-Benzoyloxy-4-[(trifluoromethyl) coumarin (7-BFC) for CYP3A4] were incubated with or without test compounds in a reaction mixture containing 1 pmol of P450 enzyme and the NADPH generating system (1.3 mM NADP, 3.54 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase and 3.3 mM MgCl₂) in potassium phosphate buffer (pH 7.4). Reactions were terminated by addition of stop solution after 45 min. Metabolite concentrations were measured with a spectrofluorometer (Molecular Device, Sunnyvale, CA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive controls (1 μM ketoconazole for CYP3A4)