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博士學位論文

Effects of Efonidipine and Aspirin on the Pharmacokinetics and Pharmacodynamics of Repaglinide in Rats

朝鮮大學校 大學院

藥學科

李 成

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흰쥐에서 에포니디핀과 아스피린이 레파그리니드의
약물동태학과 약물동력학에 미치는 영향

2012 年 2 月 24 日

朝鮮大學校 大學院

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Effects of Efonidipine and Aspirin on the Pharmacokinetics and Pharmacodynamics of Repaglinide in Rats

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국 문 초 록

흰쥐에서 에포니디핀과 아스피린이 레파그리니드의 약물동태학과 약물동력학에 미치는 영향

이 성
지 도 교 수: 최 준 식
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임상에서 당뇨병 환자에게 합병증으로 초래될 수있는 순환기 질환 예방 및 치료를 위해서 에포니디핀 또는 아스피린과 레파그리니드의 병용투여가처방되는 경우가 많다. 특히, 항고혈압제인 에포니디핀과 항혈소판제인 아스피린은 소장 및 간장에서 초회통과효과[cytochrome P450 (CYP) 3A]를 억제시킬 가능성이 있으며 더 나아가서 약물 수송과 내성에 관여하는 P-당단백질[P-glycoprotein (P-gp)]을 억제시킬 가능성이 있다. 더구나, 레파그리니드는 CYP3A 와 P-gp 의 기질이기도 하다. 따라서, 이들의 상호작용을 규명하기 위하여 흰쥐에게 레파그리니드과 에포니디핀 또는 아스피린을 병용 경구투여한 후 레파그리니드의 약물동태와 약물동력학 변수들을 대조군과 비교 검토하였다.

흰쥐에서 에포니디핀 또는 아스피린과 레파그리니드을 병용 투여시 레파그리니드의 약물동태학적 변수는 유의성 있게 변화되었다. 대조군에 비해

에포니디핀 (1.0 and 3.0 mg/kg) 또는 아스피린 (5.0 and 20.0 mg/kg)과 병용투여 후 레파그리니드의 혈장농도-시간곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 각각 유의성있게 증가되었으며, 전신클리어런스 (*CL/F*)는 각각 유의성 있게 감소되었다. 절대 생체이용률은 대조군에 비해 각각 유의성 있게 증가되었으며 상대 생체이용률은 1.13-1.51 배 증가되었다. 그리고 에포니디핀 또는 아스피린은 레파그리니드의 혈당농도를 현저하게 저하 시켰다. 정맥투여군에서는 에포니디핀 또는 아스피린은 레파그리니드의 약동학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 에포니디핀 또는 아스피린을 각각 당뇨병치료제인 레파그리니드과 병용투여 하였을 때 경구투여된 레파그리니드의 생체이용률이 유의성 있게 증가된 것은 에포니디핀 및 아스피린에 의한 신배설 감소 보다는 주로 소장에 존재하는 P-gp 억제에 의한 흡수증가와 주로 소장과 간장에 존재하는 CYP3A 억제에 의한 레파그리니드의 CYP3A (대사) 감소와 *CL/F* 의 감소에 기인한 것으로 사료되었다.

본 연구결과 임상에서 심혈관질병이 있는 당뇨병환자에게 레파그리니드와 에포니디핀 또는 아스피린을 병용투여시 이들의 상호작용출현에 대해서 주시를 해야한다고 사료된다.

Part I. Effects of Efonidipine on the Pharmacokinetics and Pharmacodynamics of Repaglinide in Rats

국문초록

항고혈압제인 에포니디핀와 레파그리니드의 병용투여가 당뇨병 환자에서 합병증으로 초래될수있는 순환기 질환 예방 및 치료를 위해서 처방되는 경우가 많다. 그러므로 이에 대한 상호작용을 알아보기 위하여 흰쥐에게 레파그리니드(경구; 0.5 mg/kg, 정맥; 0.2 mg/kg)과 에포니디핀(1.0, 3.0 mg/kg)을 병용 경구투여한 후 레파그리니드의 약물동태와 약물동력학 변수들을 대조군과 비교 검토하였다.

흰쥐에서 에포니디핀과 레파그리니드을 병용 투여시 레파그리니드의 약물동태학적 변수는 유의성 있게 변화되었다. 대조군에 비해 에포니디핀(3.0 mg/kg)과 병용투여 후 레파그리니드의 혈장농도-시간곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 각각 50.8, 31.8%로 유의성 있게 증가되었으며, 반감기 ($t_{1/2}$)과 전신클리어런스 (CL/F)는 유의성 있게 각각 변화되었다. 절대적 생체이용률은 대조군에 비해 각각 유의성 있게 증가되었으며 상대적 생체이용률은 1.17-1.51 배 증가되었다. 그리고 에포니디핀은 레파그리니드의 혈당농도는 현저하게 저하 시켰다. 정맥투여군에서는 에포니디핀은 레파그리니드의 약동학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 에포니디핀을 각각 당뇨병치료제인 레파그리니드과 병용투여

하였을 때 경구투여된 레파그리니드의 생체이용률이 유의성 있게 증가된 것은 에포니디핀에 의해서 신배설 감소 보다는 주로 소장에 존재하는 P-gp 억제에 의한 흡수증가와 주로 소장과 간장에 존재하는 CYP3A 억제에 의한 레파그리니드의 CYP3A (대사) 감소와 *CL/F* 의 감소에 기인한 것으로 사료된다.

본 연구결과 임상에서 심혈관질병이 있는 당뇨병환자에게 레파그리니드와 에포니디핀을 병용투여시 이들의 상호작용출현에 대해서 주시를 해야한다고 사료된다.

1. Introduction

Type 2 diabetes, or non-insulin-dependent diabetes mellitus (NIDDM), is a heterogeneous disorder characterized by impaired insulin secretion and insulin resistance [1, 2]. Although basal insulin secretion may remain within the normal range, insulin resistance causes chronically elevated fasting glucose levels, while a delayed and blunted postprandial insulin release causes severe postprandial hyperglycemia [3].

Type 2 diabetes mellitus is a common disease with substantial associated morbidity and mortality [4, 5]. Most adverse diabetes outcomes are a result of vascular complications, both at a macrovascular level (coronary artery disease, cerebrovascular disease, or peripheral vascular disease) and a microvascular level (retinopathy, nephropathy, or neuropathy) [6]. Macrovascular complications are more common; up to 80% of patients with type 2 diabetes will develop or die of macrovascular disease [7-15], and the costs associated with macrovascular disease are an order of magnitude greater than those associated with microvascular disease [16].

Because diabetes is defined by blood glucose levels, much of the attention in diabetes care focuses on the management of hyperglycemia. This has been magnified by a causal link between hyperglycemia and microvascular outcomes [6, 17]. However, while some observational evidence suggests that the level of glycemia is a risk factor for macrovascular disease [18-21], experimental studies to date have not clearly shown a causal relationship between improved glycemic control and reductions in serious cardiovascular outcomes [6, 17]. Given these results and the epidemiologic characteristics of diabetes complications, it would seem more logical to focus diabetes care on prevention of macrovascular

complications rather than on glucose control and microvascular complications [22]. Indeed, the importance of preventing the macrovascular complications of type 2 diabetes has started to receive greater attention. In particular, several trials have examined the benefit of managing highly prevalent risk factors, such as hypertension [22]. Hypertension is extremely common in patients with type 2 diabetes, affecting up to 60% [5], and there are a growing number of pharmacologic treatment options.

Repaglinide [(S)-(+)-2-ethoxy-4-(2-oxo-2-[(α-isobuty1-2-piperidinobenzyl) amino]ethyl) -benzoic acid, a carbamoylmethyl benzoic acid (CMBA) derivative] is a novel, fast-acting prandial oral hypoglycemic agent developed for the treatment of patients with type 2 diabetes whose disease can not be controlled by diet and exercise alone [23]. It can reduce the fasting glucose concentrations in patients with type 2 diabetes mellitus. It helps to control blood glucose by increasing the amount of insulin released by the pancreas [24]. Repaglinide stimulates the release of insulin from the pancreatic beta cells by binding to and closing ATP-dependent potassium channels. This depolarizes the plasma membrane, leading to the opening of voltage-dependent calcium channels. The influx of calcium ions, which increases intracellular Ca^{2+}, triggers exocytosis of insulin [25].

Repaglinide is rapidly absorbed from the gastrointestinal tract after oral administration. It differs from other antidiabetic agents in its structure, binding profile, duration of action and mode of excretion [26]. Repaglinide is primarily metabolized via oxidative biotransformation involving the hepatic microsomal cytochrome P450 system, particularly CYP3A4 [27]. The metabolic pathway of repaglinide involves two major sites for the principal biotransformation: the piperidine ring and the aromatic carboxylic acid group [28]. Repaglinide has affinity for P-gp and it can significantly contribute to potential drug-drug

interactions with other P-gp substrates or inhibitors [29]. Kajosaari et al. [30] reported that co-administration of repaglinide with the known P-gp inhibitor cyclosporine A could significantly increase the plasma concentrations of repaglinide in humans.

Efonidipine is a novel dihydropyridine calcium antagonist and is a powerful vasodilator with considerably less negative inotropic action than any other dihydropyridine derivative [31, 32]; its antihypertensive action is both very slow in onset and long-lasting [33]. Previous studies have shown that, in contrast to other dihydropyridines, efonidipine decreases the heart rate in patients with essential hypertension [34, 35]. In general, 1,4-dihydropyridine calcium antagonists are inhibitors of CYP3A isoforms; however, there is few report about CYP enzyme and P-gp activity for efonidipine. Therefore, we evaluated the inhibition of CYP enzyme activity and P-gp activity by efonidipine using CYP inhibition assays and rhodamine-123 retention assays in P-gp-overexpressing MCF-7/ADR cells.

Hypertension can be induced by the complications of diabetes, and repaglinide and efonidipine can be clinically prescribed for treatment of cardiovascular disease as a complication of diabetes. However, pharmacokinetic interaction between repaglinide and efonidipine has not been reported. Therefore, the present study aims to investigate the effect of efonidipine on CYP enzyme activity, P-gp activity and the pharmacokinetics and pharmacodynamics of repaglinide after oral and intravenous administration in rats.

2. Materials and Methods

2.1 Materials

Repaglinide and indomethacin (internal standard) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Efonidipine was purchased from Greencross Co. (Yongin, Gyungi, Korea). HPLC-grade methanol and acetonitrile were acquired from Merck Co. (Darmstadt, Germany). All other chemicals for this study were of reagent grade and were used without further purification. Dulbecco's modified Eagle's medium was from Hyclone Laboratories (Logan, UT, USA), Hank's balanced salt solution was from Invitrogen (Grand Island, NY, USA), fetal bovine serum was from PAA Laboratories (Etobiroke, Ontario, Canada), rhodamine was from Calbiochem, (San Diego, CA, USA) and the CYP inhibition assay kit was from GENTEST (Woburn, MA, USA).

Apparatuses used in this study included an HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus auto sampler and a Waters™ 2487 scanning UV detector (Waters Co., Milford, MA, USA), an HPLC column temperature controller (Phenomenex Co., CA, USA), a Bransonic® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Inc., NY, USA), a high-speed microcentrifuge (Hitachi Co., Tokyo, Japan), and the Onetouch® Ultra™ Blood Glucose Monitoring System (LifeScan Inc., CA, USA).

2.2 Animal studies

Male Sprague-Dawley rats (weighing 270–300 g) were purchased from the Dae Han Laboratory Animal Research Co. (Choongbuk, Korea) and were given access to a

commercial rat chow diet (No. 322-7-1, Superfeed Co., Gangwon, Korea) and tap water. The animals were housed, two per cage, and maintained at 22 ± 2 °C and 50–60% relative humidity under a 12: 12 h light:dark cycle. The experiments were initiated after acclimation under these conditions for at least 1 week. The Animal Care Committee of Chosun University (Gwangju, Korea) approved the design and the conduct of this study. The rats were fasted for at least 24 h prior to the experiments and each animal was anaesthetized lightly with ether. The left femoral artery and vein were cannulated using polyethylene tubing (SP45, i.d. 0.58 mm, o.d. 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and i.v. injection, respectively.

2.3 Drug administration

The rats were divided into six groups (n = 6, each): an oral control group (0.5 mg/kg of repaglinide dissolved in distilled water, 1.0 ml/kg) without or with 1.0 and 3.0 mg/kg of efonidipine (mixed in distilled water, 1.0 ml/kg), and an i.v. control group (0.2 mg/kg of repaglinide, dissolved in 0.9% NaCl solution, 1.5 ml/kg) without or with 1.0 and 3.0 mg/kg of efonidipine (mixed in distilled water, 1.0 ml/kg). Oral repaglinide was administered intragastrically using an injector equipped with a long blunt needle (feeding tube), and efonidipine was administered in the same manner 30 min prior to the oral administration of repaglinide. Repaglinide for i.v. administration was injected through the femoral vein within 0.5 min. A 0.4-ml aliquot of blood was collected into heparinized tubes from the femoral artery at 0.25, 0.5, 0.75, 1, 2, 4, 6, and 10 h after repaglinide oral administration and at 0 (to serve as control), 0.1, 0.25, 0.5, 1, 2, 6, and 10h after repaglinide i.v. administration. The blood samples were centrifuged at 13000 rpm for 3 min, and the plasma samples were

stored at –40 °C until HPLC analysis. Approximately 1.0 ml of whole blood collected from untreated rats was infused via the femoral artery at 0.75, 2 and 6 h to replace the blood lost due to blood sampling.

2.4 HPLC assay

The plasma concentration of repaglinide was determined by HPLC as reported by Ruzilawati et al. [36] with a slight modification. Briefly, a 50-µl aliquot of 1 µg/ml indomethacin, as an internal standard, and a 0.2-ml aliquot of 0.1 mol/l potassium dihydrogen orthophosphate (KH_2PO_4, pH 5.9) were mixed with a 0.2-ml aliquot of the plasma sample. After the mixture was vortexed, 1 ml of ethylacetate, 50 µl of isoamylalcohol and 35 µl of 1.0 M NaOH were added. The resulting mixture was then vortex-mixed for 10 min and centrifuged at 9000 rpm for 10 min. After centrifugation, the ethylacetate phase was transferred to a clean test tube and evaporated under a gentle stream of nitrogen gas at 45°C. The dried extract was reconstituted with 150 µl of mobile phase, vortex-mixed and transferred to a clean autosampler vial. A 70-µl aliquot of the supernatant was injected into the HPLC system. Chromatographic separation was achieved using a µBondapak™ C_{18} column (3.9×300 mm i.d., 10 µm, Waters Co.) attached to a µBondapak™ C18 guard column (3.9×20 mm i.d., 10 µm, Waters Co.). The mobile phase consisted of acetonitrile: 0.01 M ammonium formate (pH 2.7, adjusted with phosphoric acid) (56: 44, v/v). The flow-rate of the mobile phase was maintained at 1.0 ml/min. Chromatography was performed at 25 °C and regulated by an HPLC column temperature controller. The UV detector was operated at a wavelength of 244 nm. Plasma calibration curves were prepared and assayed in triplicate on three different days to evaluate linearity,

precision, accuracy, recovery, limit of quantitation (LOQ), limit of detection (LOD), selectivity and stability.

2.5 Determination of blood glucose concentrations

Blood samples were collected at 0, 1, 3, 6, and 10 h after oral and intravenous repaglinide administration. Blood glucose concentrations were measured immediately using the Onetouch® Ultra™ Blood Glucose Monitoring System and the glucose assay kit from BioAssay Systems (QuantiChrom™ Glucose Assay Kit) as described by Yoon and Mekalanos [37].

2.6 CYP inhibition assay

The inhibition assays of human CYP3A4 enzyme activity were performed in a multiwell plate using the CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously [38]. Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrate [7-Benzyloxy-4-(trifluoromethyl) couamrin, 7-BFC for CYP3A4] was incubated (2 μM of both CYP isoforms and 50 mM of 7-BFC were used in the incubation mixture as described in the manual of CYP inhibition assay kit) with or without efonidipine in enzyme/substrate buffer consisting of 1 pmol of P450 enzyme and a 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 $MgCl_2$) in potassium phosphate buffer (pH 7.4). Reactions were terminated by adding stop solution after 45-min incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. A positive control (1 μM

ketoconazole) was run on the same plate and produced 99% inhibition. All experiments were performed in duplicate, and results are expressed as the percent of inhibition.

2.7 Rhodamine-123 retention assay

The procedures used for the Rho-123 retention assay were similar to a previously reported method [39]. The P-gp overexpressed multidrug resistant human breast carcinoma cell line (MCF-7/ADR cells) were seeded in 24-well plates at a seeding density of 10^5 cells and preincubated with efonidipine and verapamil for 30 min. At 80% confluency, the cells were incubated in fetal bovine serum free Dulbecco's modified Eagle's medium for 18 h. The culture medium was changed to Hanks' balanced salt solution and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20 μM rhodamine-123 in the presence or absence of efonidipine (1, 3 and 10 μM) for 90 min, the medium was completely removed. The cells were then washed three times with ice-cold phosphate buffer (pH 7.0) and lysed in lysis buffer. Rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and are presented as the ratio to control.

2.8 Pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using Thermo Kinetica Software Version 5.0 (Thermo Fisher Scientific Inc., Miami, OK, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of repaglinide concentration during the elimination phase. The terminal half-life ($t_{1/2}$) was calculated by

$0.693/K_{el}$. The peak plasma concentration (C_{max}) and time to reach peak plasma concentration (T_{max}) of repaglinide in plasma were obtained by visual inspection of the data from the concentration–time curve. The area under the plasma concentration-time curve from 0 h to the time (AUC_{0-t}) of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC from zero to infinity ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. The mean residence time (MRT) was calculated by dividing the first moment of AUC ($AUMC_{0-\infty}$) by $AUC_{0-\infty}$. The apparent volume of distribution at steady state (Vd_{ss}) was estimated by the product of $MRT_{i.v.}$ and CL_t after i.v. dosing. Total body clearance (CL/F) was calculated by:

$$CL/F = \frac{Dose}{AUC}$$

The absolute bioavailability (A.B.) was calculated by:

$$AB\% = \frac{AUC_{oral}}{AUC_{i.v.}} \times \frac{Dose_{i.v.}}{Dose_{oral}} \times 100\%$$

The relative bioavailability (R.B.) was calculated by:

$$RB\% = \frac{AUC_{with\ efonidipine}}{AUC_{control}} \times 100\%$$

2.9 Statistical analysis

Statistical analysis was conducted using one-way ANOVA followed by a posteriori testing with the Dunnett correction. Differences were considered significant at $P < 0.05$. All mean values are presented with their standard deviation (Mean ± SD).

3. Results

3.1 Chromatographic separation

Figure 1 illustrates chromatograms of the blank rat plasma samples (A) and the rat plasma spiked with repaglinide, indomethacin (internal standard, IS) (B), and efonidipine (C). The retention times of repaglinide and indomethacin (IS) were 6.8 and 8.5 min, respectively. The overall run time lasted 10 min. Efonidipine did not interfere with the detection of repaglinide and indomethacin (IS) in this method.

3.2 Validation characteristics of analysis

Figure 2 shows the calibration curve of repaglinide constructed by plotting the ratio of the peak area of repaglinide to that of indomethacin as a function of the plasma repaglinide concentrations (50, 100, 200, 500, 1000, 2000 and 5000 ng/ml). There was excellent linearity over the range of 50–5000 ng/ml with a mean correlation coefficient of 0.999. The typical equation describing the calibration curve in rat plasma was $y = 0.0031\ x - 0.0052$, where y is the peak area ratio of repaglinide to indomethacin and x is the concentration of repaglinide. The recovery of repaglinide in rat plasma was 102.2%. The LOD and LOQ for repaglinide in rat plasma were 20 ng/ml. The intra- and inter-day coefficients of variation for repaglinide were below 12.1 and 11.5%, respectively.

3.3 CYP inhibition assay

The inhibitory effect of ketoconazole and efonidipine on CYP3A4 enzyme activity is

shown in Figure 3. Ketoconazole and efonidipine inhibited CYP3A4 enzyme activity in a concentration-dependent manner. Ketoconazole and efonidipine strongly inhibited CYP3A4 enzyme with IC_{50} values of 0.03 and 0.08 μM.

3.4 Rhodamine-123 retention assay

As shown in Figure 4, accumulation of rhodamine-123, a P-gp substrate, was raised in MCF-7/ADR cells overexpressing P-gp compared to that in MCF-7 cells lacking P-gp. The concurrent use of efonidipine enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner and the uptake was significantly ($P < 0.01$) increased at concentrations ranging from 1 to 10 μM. This result suggests that efonidipine significantly inhibits P-gp activity.

3.5 Determination of blood glucose concentrations

Repaglinide pharmacodynamics was characterized by determining blood glucose levels. Mean blood glucose–time profiles in rats after oral (0.5 mg/kg) and intravenous (0.2 mg/kg) administration of repaglinide in the presence or absence of efonidipine (1.0 and 3.0 mg/kg) (n = 6, each) are shown in Figure 5 and 6. Compared to the control group, efonidipine significantly decreased the blood glucose levels after oral administration of repaglinide, revealing a significant hypoglycemic effect (Figure 5); however, there was no significant difference after intravenous administration of repaglinide (Figure 6).

3.6 Effect of efonidipine on the pharmacokinetics of oral repaglinide

Mean arterial plasma concentration-time profiles of repaglinide following oral

administration of repaglinide (0.5 mg/kg) to rats in the presence or absence of efonidipine (1.0 and 3.0 mg/kg) are shown in Figure 7; the corresponding pharmacokinetic parameters are shown in Table 1. Efonidipine significantly altered the pharmacokinetic parameters of repaglinide. Compared to the control group (given oral repaglinide alone), efonidipine significantly increased the area under the plasma concentration-time curve ($AUC_{0-\infty}$, $P < 0.01$ at 3.0 mg/kg) and the peak plasma concentration (C_{max}, $P < 0.05$ at 3.0 mg/kg) of repaglinide by 50.8% and 31.8%, respectively. The terminal half-life ($t_{1/2}$) was significantly increased (3.0 mg/kg, $P < 0.05$) and the total body clearance (CL/F) was significantly decreased (3.0 mg/kg, $P < 0.01$) by efonidipine. Efonidipine also increased the absolute bioavailability (*A.B.*) of repaglinide by 50.6% (3.0 mg/kg, $P < 0.01$) compared to the oral control group (32.2%), and the relative bioavailability (*R.B.*) of repaglinide by 1.17- to 1.51-fold. There was no significant difference in the time to reach peak plasma concentration (T_{max}) of repaglinide in the presence of efonidipine.

3.7 Effect of efonidipine on the pharmacokinetics of intravenous repaglinide

Mean plasma concentration-time profiles of repaglinide after the intravenous administration of repaglinide (0.2 mg/kg) to rats in the presence or absence of efonidipine (1.0 and 3.0 mg/kg) are shown in Figure 8; the correlated pharmacokinetic parameters are shown in Table 2. Compared to the control group, efonidipine (3.0 mg/kg) significantly ($P < 0.05$) increased the $AUC_{0-\infty}$ (31.8%) of repaglinide. The CL_t and Vd_{ss} values of repaglinide tended to decrease, but this trend was not statistically significant. The $t_{1/2}$ of repaglinide was also increased, but this increase was not significant.

Accordingly, while there was no significant change in the intravenous pharmacokinetics

of repaglinide, the enhanced oral bioavailability in the presence of efonidipine could be mainly due to inhibition of P-gp in the small intestine and to inhibition of CYP3A isoforms in the small intestine and/or liver by efonidipine rather than to reduction of renal elimination of repaglinide.

4. Discussion

The cytochrome P450 superfamily is a large and diverse group of enzymes. Most CYP enzymes function to catalyze the oxidation of organic substances. The substrates of CYP enzymes include metabolic intermediates such as lipids and steroidal hormones, as well as xenobiotic substances such as drugs and other toxic chemicals. CYPs are the major enzymes involved in drug metabolism and bioactivation, accounting for about 75% of the total number of different metabolic reactions [40].

Many drugs may increase or decrease the activity of various CYP isozymes either by inducing the biosynthesis of an isozyme (enzyme induction) or by directly inhibiting the activity of the CYP (enzyme inhibition). This is a major source of adverse drug interactions, since changes in CYP enzyme activity may affect the metabolism and clearance of various drugs. For example, if one drug inhibits the CYP-mediated metabolism of another drug, the second drug may accumulate within the body to toxic levels. Hence, these drug interactions may necessitate dosage adjustments or the use of drugs that do not interact with the CYP system. Such drug interactions are especially important to take into account when using drugs of vital importance to the patient, drugs with important side-effects and drugs with small therapeutic windows, but any drug may be subject to an altered plasma concentration due to altered drug metabolism.

One of the principal cytochrome P450 isoforms involved in repaglinide metabolism in human liver microsomes is CYP3A4. CYP3A4 is the most abundant isoform of cytochrome P450 in the human liver [41] and is responsible for the metabolism of many different drugs [42]. Levels of CYP3A4 vary substantially between individuals; these variations may be

related to induction of the isoform by various drugs and may partially account for the observed inter-individual variations in repaglinide metabolism [43].

P-glycoprotein is a well-characterized ATP-binding cassette (ABC)-transporter (which transports a wide variety of substrates across extra- and intra-cellular membranes) of the multi-drug resistance (MDR) subfamily [44]. P-gp is extensively distributed and expressed in the intestinal epithelium, hepatocytes, renal proximal tubular cells, adrenal gland and capillary endothelial cells comprising the blood-brain and blood-testis barrier. In addition, P-gp is an ATP-dependent drug efflux pump for xenobiotic compounds and has broad substrate specificity.

P-gp is expressed with CYP3A isoforms and glutathione-S-transferase [45, 46], which may have synergistic functions in regulating the bioavailability of many orally ingested compounds. In the small intestine, P-gp is co-localized with CYP3A isoforms at the apical membrane of the cells [47]. P-gp and CYP3A isoforms might act synergistically to limit oral absorption and first-pass metabolism [48]. Poor solubility and first-pass metabolism in the liver and epithelial cells of the small intestine result in low bioavailability of repaglinide (56%) [29]. Considering that repaglinide is a substrate of both CYP3A and P-gp [27, 29], CYP3A and P-gp inhibitors might alter the bioavailability and pharmacokinetics of repaglinide. The inhibitory effect of efonidipine against CYP3A isoforms-mediated metabolism was confirmed through the use of recombinant CYP3A isoforms.

As shown in Figure 3, efonidipine inhibited CYP3A4 enzyme activity with an IC_{50} value of 0.08 μM. Furthermore, the cell-based assay using rhodamine-123 indicated that efonidipine (1-10 μM) significantly ($P < 0.01$) inhibited P-gp-mediated drug efflux (Figure 4). Therefore, the pharmacokinetic characteristics of repaglinide were evaluated in the

absence and presence of efonidipine in rats. As CYP3A9 in rat is the ortholog of CYP3A4 in human [49], rat CYP3A2 is also similar to human CYP3A4 [50, 51]. Human 3A4 and rat 3A1 have 73% protein homology [52]. Rats were therefore selected as an animal model in this study to evaluate the potential pharmacokinetic interactions mediated by CYP3A4, although there are almost certainly some differences in enzyme activity between rat and human [53]. Nevertheless, efonidipine might increase absorption of repaglinide in the intestine through the inhibition of P-gp and CYP3A isoforms.

In this study, efonidipine significantly decreased blood glucose levels compared to the oral control group and increased the $AUC_{0-\infty}$ by 50.8% and the *R.B.* of repaglinide by 1.17- to 1.51-fold. Because efonidipine considerably increased the C_{max} and $t_{1/2}$ of repaglinide, it seems that efonidipine inhibited the CYP3A isoforms-mediated metabolism of repaglinide mainly during first-pass metabolism. CYP3A isoforms are present in considerable quantities in the small-intestinal mucosa [54, 55], and the intestinal CYP3A isoforms have been shown to play a major role in drug interactions with CYP3A inhibitors. These results are consistent with a report by Niemi et al. [56] showing that clarithromycin, a CYP3A4 inhibitor, significantly increased the $AUC_{0-\infty}$ and C_{max} of repaglinide and enhanced the repaglinide blood glucose-lowering effect, despite the fact that no statistically significant differences were found in the blood concentrations between control group and clarithromycin. Niemi et al. [57] reported that gemfibrozil significantly increased the $AUC_{0-\infty}$ of repaglinide 8.1-fold and enhanced the blood glucose-lowering effect of repaglinide, while Kajosaari et al. [30] also reported that co-administration of repaglinide with the known P-gp inhibitor cyclosporine A significantly increased the plasma concentration of repaglinide in humans. These results are consistent with our study.

After intravenous administration of repaglinide with efonidipine, the $AUC_{0-\infty}$ of repaglinide was significantly increased ($P < 0.05$) by efonidipine. The CL_t value of repaglinide tended to decrease, but was not statistically significant. This suggests that the effects of oral efonidipine on the inhibition of hepatic metabolism of repaglinide via CYP3A isoforms were almost negligible. In contrast to those of oral repaglinide, the pharmacokinetics of intravenous repaglinide were not affected by concurrent use of efonidipine. Accordingly, while there was no significant change in the pharmacokinetics of intravenous repaglinide, the enhanced oral bioavailability may be mainly due to increased intestinal absorption via P-gp inhibition by efonidipine rather than to reduced hepatic and renal elimination of repaglinide.

5. Conclusion

Efonidipine enhanced the bioavailability of oral repaglinide in this study. The enhanced bioavailability of repaglinide might be mainly due to inhibition of the CYP3A isoforms-mediated metabolism of repaglinide in the small intestine and/or liver and the inhibition of the P-gp efflux pump in the small intestine and reduction of total body clearance of repaglinide by efonidipine.

Since the present study has raised the awareness of potential drug interactions by concomitant use of efonidipine with repaglinide, the clinical significance of this finding needs to be further evaluated in clinical studies. Based on the results, if the drug interactions are confirmed in diabetes patients with cardiovascular diseases, the dosage of repaglinide should be adjusted and blood glucose concentrations should be carefully monitored when repaglinide is used concomitantly with efonidipine.

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Table 1. Mean (± SD) pharmacokinetic parameters of repaglinide after the oral administration of repaglinide (0.5 mg/kg) to rats in the presence or absence of efonidipine (1.0 and 3.0 mg/kg) (n = 6, each).

Parameter (units)	Repaglinide (Control)	Repaglinide + efonidipine	
		1.0 mg/kg	3.0 mg/kg
$AUC_{0-\infty}$ (ng·h/ml)	506 ± 81	593 ± 101	763 ± 145**
C_{max} (ng/ml)	192 ± 31	207 ± 35	253 ± 45*
T_{max} (h)	0.64 ± 0.10	0.63 ± 0.11	0.61 ± 0.12
$t_{1/2}$ (h)	2.4 ± 0.4	2.7 ± 0.5	3.0 ± 0.6*
CL/F (ml/min/kg)	15.3 ± 2.7	13.1 ± 2.2	10.0 ± 1.7**
A. B. (%)	32.2 ± 5.2	37.7 ± 6.4	48.5 ± 9.1**
R. B. (%)	100	117	151

* $P < 0.05$, ** $P < 0.01$, significant difference compared to the control group given repaglinide alone.

$AUC_{0-\infty}$: 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; *CL/F*: total body clearance; *A.B.*: absolute bioavailability; *R.B.*: relative bioavailability.

Table 2. Mean (± SD) pharmacokinetic parameters of repaglinide after the intravenous administration of repaglinide (0.2 mg/kg) to rats in the presence or absence of efonidipine (1.0 and 3.0 mg/kg) (n = 6, each).

Parameter (units)	Repaglinide (Control)	Repaglinide + efonidipine	
		1.0 mg/kg	3.0 mg/kg
$AUC_{0-\infty}$ (ng·h/ml)	629 ± 101	717 ± 122	829 ± 158*
$t_{1/2}$ (h)	2.5 ± 0.4	2.6 ± 0.4	2.9 ± 0.6
CL_t (ml/min/kg)	5.2 ± 0.8	4.5 ± 0.7	3.9 ± 0.6
Vd_{ss} (ml/kg)	13.2 ± 2.2	12.3 ± 2.0	11.6 ± 2.0

* $P < 0.05$, significant difference compared to the control group given repaglinide alone.

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; $t_{1/2}$: terminal half-life; CL_t: total body clearance; Vd_{ss}: volume of distribution at steady state.

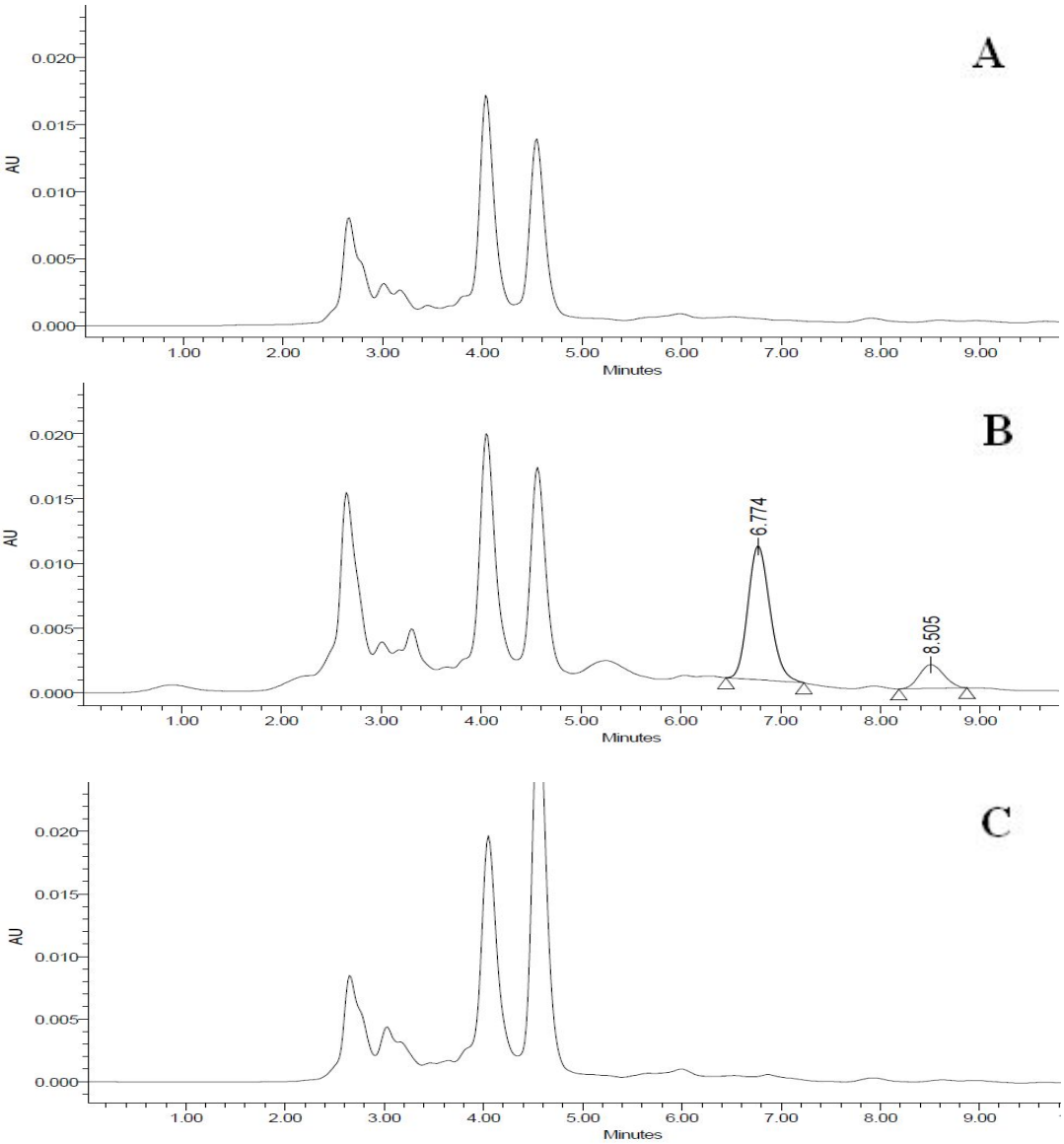


Figure 1. HPLC chromatograms of the blank rat plasma (A), the plasma spiked with repaglinide (6.8 min), indomethacin (internal standard; 8.5 min) (B), and efonidipine in the rat plasma (C).

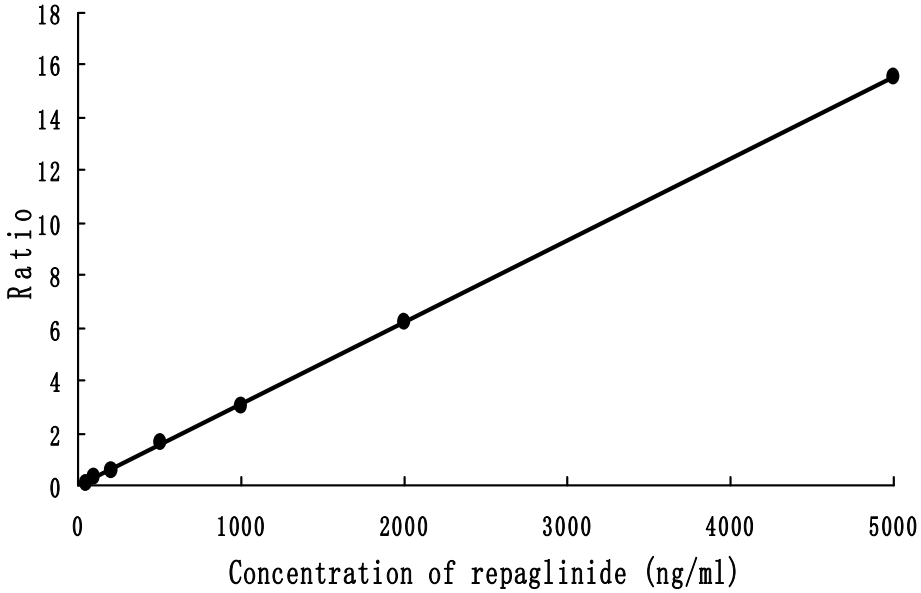
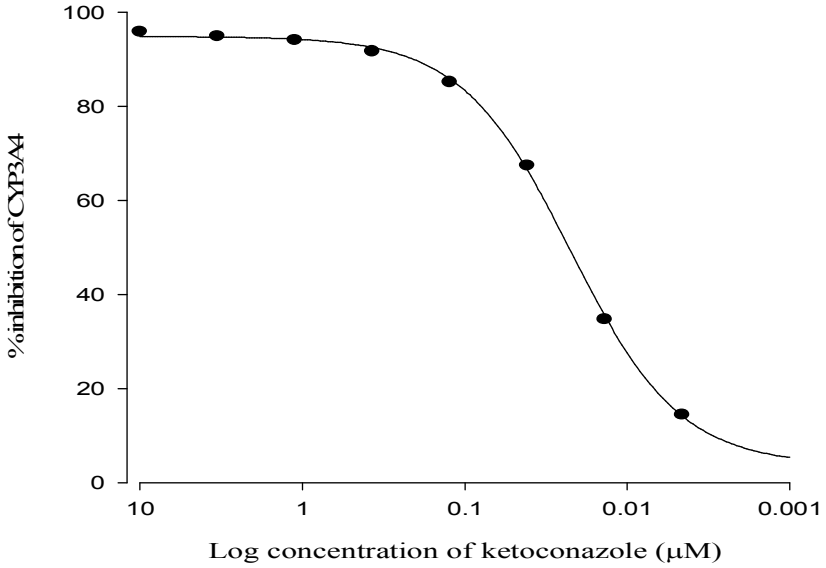


Figure 2. A calibration curve of repaglinide was linear in the concentration range of 50–5000 ng/ml in rat plasma. The typical equation describing the calibration curve in rat plasma was $y = 0.0031\ x - 0.0052$, where "y" is the peak area ratio of repaglinide to indomethacin and "x" is the concentration of repaglinide.



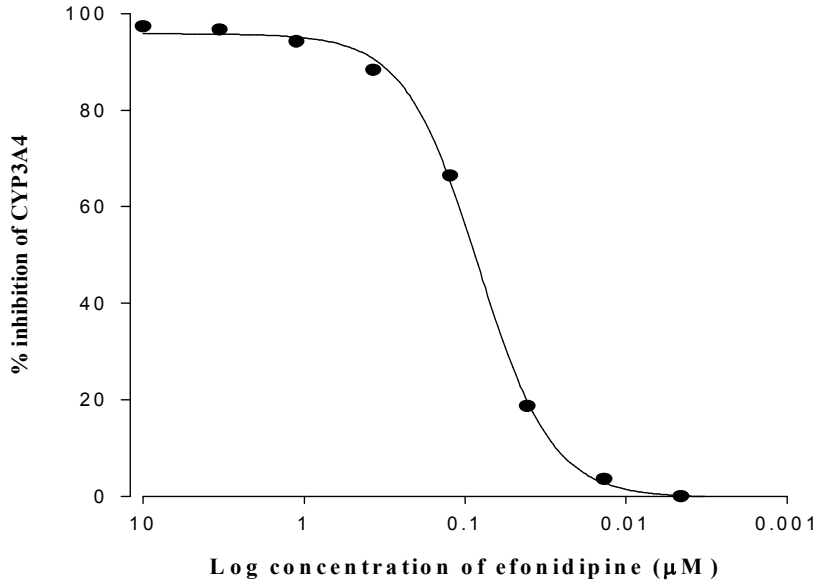


Figure 3. Inhibitory effects of ketoconazole and efonidipine on CYP3A4 activity. The experiment was performed in duplicate, and the result is expressed as the percent of inhibition.

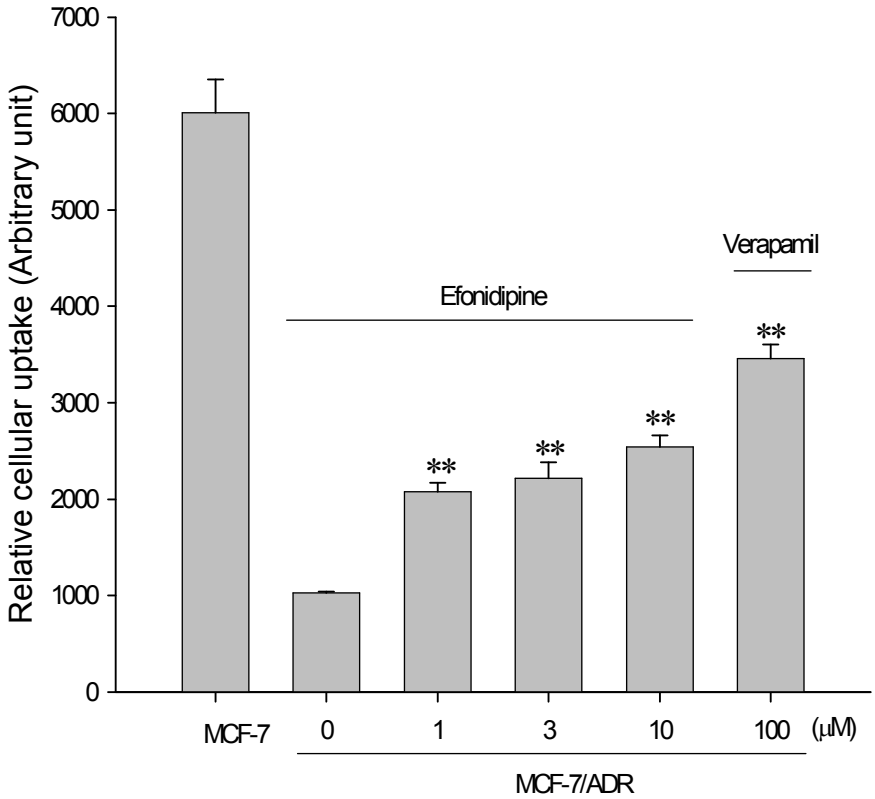


Figure 4. Rhodamine-123 retention. MCF-7/ADR cells were preincubated with efonidipine for 30 min after incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min. Data represents mean ± SD of 6 separate samples. Verapamil (100 μM) was used as a positive control (significant versus the control MCF-7 cells, $^{**}P < 0.01$).

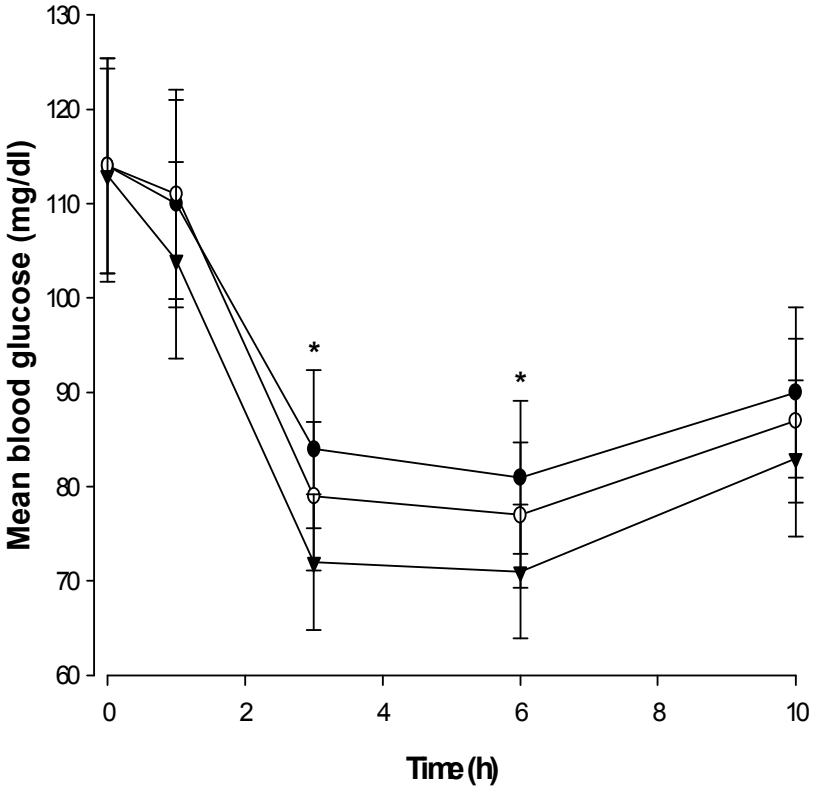


Figure 5. Mean blood glucose–time profiles in rats after oral (0.5 mg/kg) administration of repaglinide in the presence or absence of efonidipine (1.0 and 3.0mg/kg) (n = 6, each). Bars represent the standard deviation; Oral administration of 0.5 mg/kg repaglinide (control) (*solid circles* ●), with 1.0 mg/kg efonidipine (*open circles* ○) and with 3.0 mg/kg efonidipine (*solid triangles* ▼). *$P < 0.05$ significant difference compared to control.

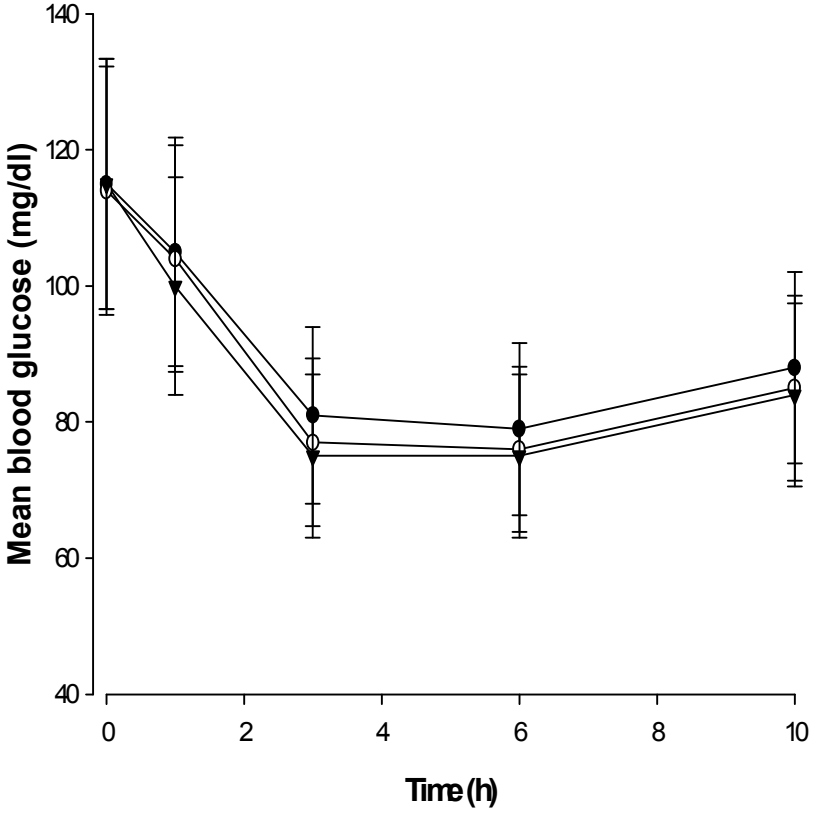


Figure 6. Mean blood glucose–time profiles in rats after intravenous (0.2 mg/kg) administration of repaglinide in the presence or absence of efonidipine (1.0 and 3.0 mg/kg) (n = 6, each). Bars represent the standard deviation; Intravenous administration of 0.2 mg/kg repaglinide (control) (*solid circles* ●), with 1.0 mg/kg efonidipine (*open circles* ○) and with 3.0 mg/kg efonidipine (*solid triangles* ▼).

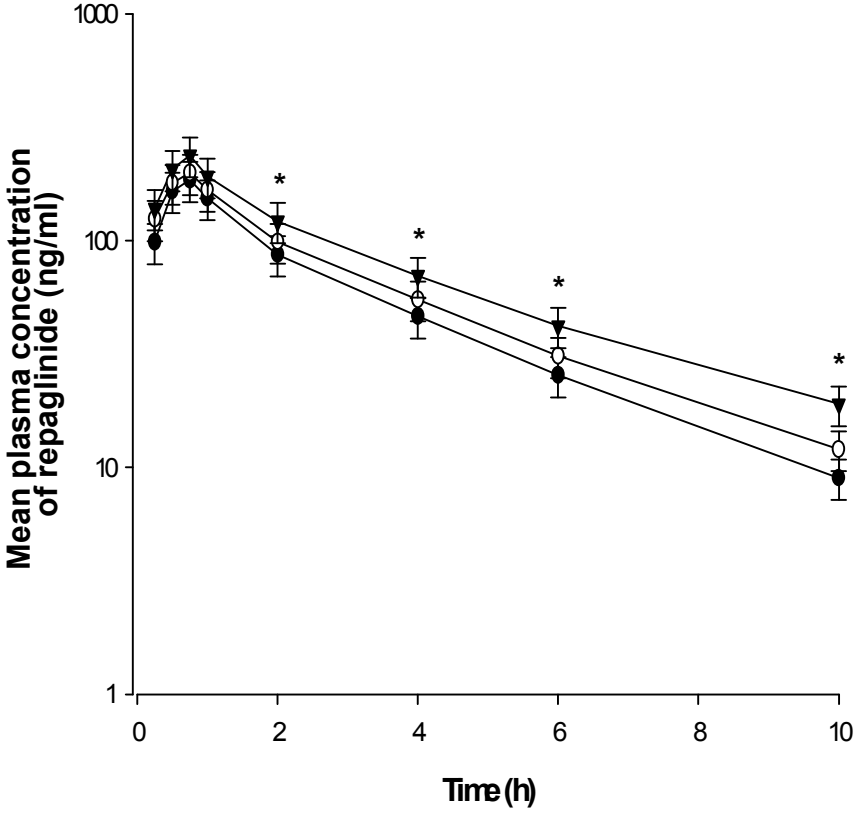


Figure 7. Mean plasma concentration–time profiles of repaglinide after oral (0.5 mg/kg) administration of repaglinide to rats in the presence or absence of efonidipine (1.0 and 3.0 mg/kg) (n = 6, each). Bars represent the standard deviation; Oral administration of 0.5 mg/kg repaglinide (control) (*solid circles* ●), with 1.0 mg/kg efonidipine (*open circles* ○) and with 3.0 mg/kg efonidipine (*solid triangles* ▼). *$P < 0.05$ significant difference compared to control.

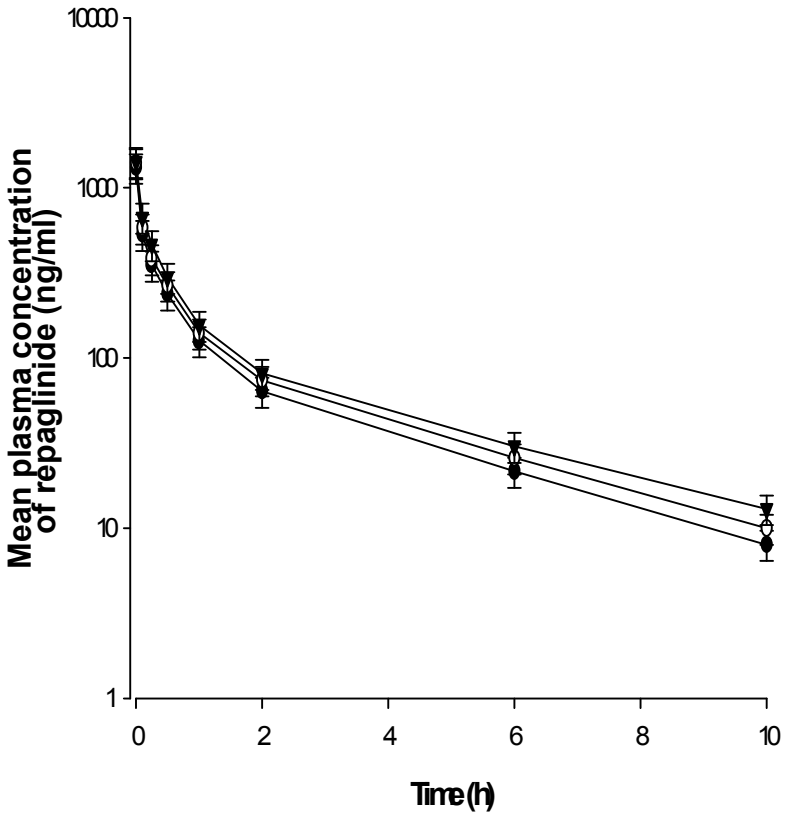


Figure 8. Mean plasma concentration–time profiles of repaglinide after intravenous (0.2 mg/kg) administration of repaglinide to rats in the presence or absence of efonidipine (1.0 and 3.0 mg/kg) (n = 6, each). Bars represent the standard deviation; Intravenous administration of 0.2 mg/kg repaglinide (control) (*solid circles* ●), with 1.0 mg/kg efonidipine (*open circles* ○) and with 3.0 mg/kg efonidipine (*solid triangles* ▼).

Abstract

The purpose of this study was to investigate the effects of efonidipine on the pharmacokinetics and pharmacodynamics of repaglinide in rats. Hypertension can be induced by the complications of diabetes. In the clinic, efonidipine can be prescribed to treat cardiovascular disease as a complication of diabetes; however, repaglinide and efonidipine interact with the same cytochrome P450 (CYP) enzyme and P-glycoprotein (P-gp).

The effect of efonidipine on P-gp and CYP enzyme activity was evaluated. The pharmacokinetic parameters of repaglinide and blood glucose concentrations were also determined in rats after oral (0.5 mg/kg) and intravenous (0.2 mg/kg) administration of repaglinide to rats in the presence and absence of efonidipine (1.0 and 3.0 mg/kg).

Efonidipine inhibited CYP3A4 activity with IC_{50} value of 0.08 μM, and efonidipine significantly inhibited P-gp activity in a concentration-dependent manner. Compared to the oral control group, efonidipine significantly increased the area under the plasma concentration-time curve ($AUC_{0-\infty}$) ($P < 0.01$ for 3.0 mg/kg) and the peak plasma concentration (C_{max}) ($P < 0.05$ for 3.0 mg/kg) of repaglinide by 51.3% and 28.6%, respectively. The terminal half-life ($t_{1/2}$) was significantly increased (3.0 mg/kg, $P < 0.05$) and the total body clearance (CL/F) was significantly decreased (3.0 mg/kg, $P < 0.01$) by efonidipine. Efonidipine also significantly ($P < 0.01$ for 3.0 mg/kg) increased the absolute bioavailability (*A.B.*) of repaglinide by 51.5% compared to the oral control group (33.6%). Moreover, the relative bioavailability (*R.B.*) of repaglinide was 1.17- to 1.51-fold greater than that of the control group. Compared to the intravenous control, efonidipine significantly ($P < 0.05$ for 3.0 mg/kg) increased the plasma concentration-time curve ($AUC_{0-\infty}$) of

intravenously administered repaglinide. The blood glucose concentrations were significantly different compared to the oral control groups.

Efonidipine enhanced the oral bioavailability of repaglinide, and this effect may be mainly attributable to inhibition of the CYP3A isoforms-mediated metabolism of repaglinide in the small intestine and/or liver and to inhibition of the P-gp efflux pump in the small intestine rather than to reduction of elimination of repaglinide by efonidipine. The present study raises the awareness of potential drug interactions by concomitant use of efonidipine with repaglinide. Therefore, the concurrent use of efonidipine with repaglinide may require close monitoring of both potential drug interactions and blood glucose concentrations.

Key words: Repaglinide · Pharmacokinetics · Pharmacodynamics · Efonidipine · CYP3A isoforms · P-gp · Rats

Part II. Effects of Aspirin on the Pharmacokinetics and Pharmacodynamics and Repaglinide in Rats

국문초록

항혈소판제인 아스피린와 레파그리니드의 병용투여가 당뇨병 환자에서 합병증으로 초래될 수있는 순환기 질환 예방 및 치료를 위해서 처방되는 경우가 많다. 그러므로 이에 대한 상호작용을 알아보기 위하여 흰쥐에게 레파그리니드(경구; 0.5 mg/kg, 정맥; 0.2 mg/kg)과 아스피린(5.0, 20.0 mg/kg)을 병용 경구투여한 후 레파그리니드의 약물동태와 약물동력학 변수들을 대조군과 비교 검토하였다.

흰쥐에서 아스피린과 레파그리니드을 병용 투여시 레파그리니드의 약물동태학적 변수는 유의성 있게 변화되었다. 대조군에 비해 아스피린 (5.0 and 20.0 mg/kg)과 병용투여 후 레파그리니드의 혈장농도-시간곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 각각 유의성 ($P < 0.05$) 있게 증가되었으며, 전신클리어런스 (CL/F)는 각각 유의성 ($P < 0.05$) 있게 감소되었다. 절대 생체이용률은 대조군에 비해 각각 유의성 있게 증가되었으며 상대 생체이용률은 1.13-1.37 배 증가되었다. 그리고 아스피린은 레파그리니드의 혈당농도를 현저하게 저하 시켰다. 정맥투여군에서는 아스피린은 레파그리니드의 약동학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 아스피린을 각각 당뇨병치료제인 레파그리니드과 병용투여하였을 때 경구투여된 레파그리니드의 생체이용률이 유의성 있게 증가된 것은 아스피린에 의한 신배설 감소 보다는 주로 소장에 존재하는 P-gp 억제에 의한 흡수증가와 주로 소장과 간장에 존재하는 CYP3A 억제에 의한 레파그리니드의 CYP3A (대사) 감소와 *CL/F* 의 감소에 기인한 것으로 사료되었다.

본 연구결과 임상에서 심혈관질병이 있는 당뇨병환자에게 레파그리니드와 아스피린을 병용투여시 이들의 상호작용출현에 대해서 주시를 해야한다고 사료된다.

1. Introduction

Repaglinide is a novel post-prandial glucose regulator for the treatment of type 2 diabetes mellitus [1, 2]. It helps to control blood glucose by increasing the amount of insulin released by the pancreas [3]. Repaglinide stimulates the release of insulin from pancreatic β-cells by binding to and closing ATP-dependent potassium channels. This depolarizes the plasma membrane, leading to the opening of voltage-dependent calcium channels. The influx of calcium ions, which increases intracellular Ca2+, triggers exocytosis of insulin [4].

Aspirin is a salicylate drug that is often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication [5]. More recently, aspirin has been evaluated in the prevention of two leading causes of death in cardiovascular disease (including stroke, myocardial infarction and thromboembolism) [6, 7]. Previous studies have recommended aspirin for primary prevention for diabetic patients at risk of recurrent cardiovascular risk [8, 9].

Diabetes mellitus type 2 – formerly non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes – is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency [10]. Diabetes is often initially managed by exercise and dietary modification. If the condition progresses, medications may be needed. Often affecting the obese, diabetes requires patients to routinely check their blood sugar. Unlike type 1 diabetes, there is very little tendency toward ketoacidosis although it is not unheard of [11]. One effect that can occur is nonketonic hyperglycemia. Long-term complications from high blood sugar include increased risk of heart attacks, strokes, amputation, diabetic retinopathy in which eyesight is affected, and

kidney failure. In extreme cases, circulation of in limbs is affected and amputation may be required. Loss of hearing, eyesight, and cognitive ability has also been linked to this condition.

Hypertension or high blood pressure is a chronic cardiac medical condition in which the systemic arterial blood pressure is elevated such that the heart has to work harder than it should to pump the blood throughout the body. Type 2 diabetes and hypertension are strongly linked. Hypertension can increase the chance of cardiovascular disease, leading to a heart attack or stroke, as well raising the risk of kidney disease, blindness and the need for amputation. Research has shown that at least 40% of people suffering from diabetes in its various forms have hypertension [12]. In people without diabetes, the endothelial cells that line blood vessels contain nitric oxide (NO), which helps to maintain blood pressure at a normal level. Unfortunately, people with diabetes experience a reduction of NO in endothelial cells caused by attacks by cell-damaging "renegade" molecules called free radicals. This process leads to endothelial dysfunction, which is frequently a harbinger of cardiovascular disease such as heart attack or stroke. High levels of fatty acids and triglycerides in the blood stream - common in persons with diabetes - may also damage the heart, leading to cardiomyopathy, which occurs when the heart muscle becomes inflamed and doesn't work properly. If neglected, this condition can eventually lead to heart failure [12].

Repaglinide is rapidly absorbed from the gastrointestinal tract after oral administration. It differs from other antidiabetic agents in its structure, binding profile, duration of action and mode of excretion [13]. Repaglinide is primarily metabolized via oxidative biotransformation involving the hepatic microsomal cytochrome P450 system, particularly

the CYP3A4 [14]. The metabolic pathway of repaglinide indicates two major sites for the principal biotransformation of repaglinide: the piperidine ring and the aromatic carboxylic acid group [15]. Repaglinide has affinity for P-gp and it can significantly contribute to potential drug-drug interactions with other P-gp substrates or inhibitors [16]. Kajosaari *et al*. [17] reported that co-administration of repaglinide with the known P-gp inhibitor cyclosporine A could significantly increase the plasma concentrations of repaglinide in humans.

Aspirin is absorbed rapidly from the gastrointestinal tract when taken orally. There are few reports about CYP enzyme and P-gp activity for aspirin. Therefore, we evaluated the inhibition of CYP enzyme and P-gp activity by aspirin using CYP inhibition assays and rhodamine-123 retention assays in P-gp-overexpressing MCF-7/ADR cells.

Clinically repaglinide and aspirin, an antiplatelet agent, can be prescribed for prevention or treatment of diabetes and cardiovascular disease as a complication of diabetes. However, pharmacokinetic interaction between repaglinide and aspirin has not been reported. Therefore, the present study aims to investigate the effect of aspirin on CYP enzyme activity, P-gp activity and the pharmacokinetics and pharmacodynamics of repaglinide after oral and intravenous administration in rats.

2. Materials and Methods

2.1 Materials

Repaglinide, aspirin and indomethacin (internal standard) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were acquired from Merck Co. (Darmstadt, Germany). All other chemicals for this study were of reagent grade and were used without further purification. Dulbecco's modified Eagle's medium was from Hyclone Laboratories (Logan, UT, USA), Hank's balanced salt solution was from Invitrogen (Grand Island, NY, USA), fetal bovine serum was from PAA Laboratories (Etobiroke, Ontario, Canada), rhodamine was from Calbiochem, (San Diego, CA, USA) and the CYP inhibition assay kit was from GENTEST (Woburn, MA, USA).

Apparatuses used in this study included an HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus auto sampler and a WatersTM 2487 scanning UV detector (Waters Co., Milford, MA, USA), an HPLC column temperature controller (Phenomenex Inc., CA, USA), a Bransonic® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., NY, USA), a high-speed microcentrifuge (Hitachi Co., Tokyo, Japan), and the Onetouch® UltraTM Blood Glucose Monitoring System (LifeScan Inc., CA, USA).

2.2 Animal studies

Male Sprague-Dawley rats (weighing 270–300 g) were purchased from the Dae Han Laboratory Animal Research Co. (Choongbuk, Korea) and were given access to a

commercial rat chow diet (No. 322-7-1, Superfeed Co., Gangwon, Korea) and tap water. The animals were housed, two per cage, and maintained at 22 ± 2°C and 50–60% relative humidity under a 12:12 h light-dark cycle. The experiments were initiated after acclimation under these conditions for at least 1 week. The Animal Care Committee of Chosun University (Gwangju, Korea) approved the design and the conduct of this study. The rats were fasted for at least 24 h prior to the experiments and each animal was anaesthetized lightly with ether. The left femoral artery and vein were cannulated using polyethylene tubing (SP45, i.d. 0.58 mm, o.d. 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and i.v. injection, respectively.

2.3 Drug administration

The rats were divided into six groups (*n*=6, each): an oral control group (0.5 mg/kg of repaglinide dissolved in distilled water, 1.0 ml/kg) without or with 5.0 and 20.0 mg/kg of aspirin (mixed in distilled water, 3.0 ml/kg), and an i.v. control group (0.2 mg/kg of repaglinide, dissolved in 0.9% NaCl solution, 1.5 ml/kg) without or with 5.0 and 20.0 mg/kg of aspirin (mixed in distilled water, 3.0 ml/kg). Oral repaglinide was administered intragastrically using an injector equipped with a long blunt needle (feeding tube), and aspirin was administered in the same manner 30 min prior to the oral administration of repaglinide. Repaglinide for i.v. administration was injected through the femoral vein within 0.5 min. A 0.5-ml aliquot of blood was collected into heparinized tubes from the femoral artery at 0.25, 0.5, 0.75, 1, 2, 4, 6, and 10 h after repaglinide oral administration and at 0 (to serve as control), 0.1, 0.25, 0.5, 1, 2, 6, and 10h after repaglinide i.v. administration. The blood samples were centrifuged at 13000 rpm for 3 min, and the plasma samples were

stored at –40°C until HPLC analysis. Approximately 1.0 ml of whole blood collected from untreated rats was infused via the femoral artery at 0.75, 2, 4 and 6 h to replace the blood lost due to blood sampling.

2.4 HPLC assay

The plasma concentration of repaglinide was determined by HPLC as reported by Ruzilawati *et al*. [4] with a slight modification. Briefly, a 50-µl aliquot of 1 µg/ml indomethacin, as an internal standard, and a 0.2-ml aliquot of 0.1 mol/l potassium dihydrogen orthophosphate (KH2PO4, pH 5.9) were mixed with a 0.2-ml aliquot of the plasma sample. After the mixture was vortexed, 1 ml of ethylacetate, 50 µl of isoamylalcohol and 35 µl of 1.0 M NaOH were added. The resulting mixture was then vortex-mixed for 10 min and centrifuged at 9000 rpm for 10 min. After centrifugation, the ethylacetate phase was transferred to a clean test tube and evaporated under a gentle stream of nitrogen gas at 45°C. The dried extract was reconstituted with 150 µl of mobile phase, vortex-mixed and transferred to a clean autosampler vial. A 70-µl aliquot of the supernatant was injected into the HPLC system. Chromatographic separation was achieved using a µBondapakTM C18 column (3.9×300 mm i.d., 10 µm, Waters Co.) attached to a µBondapakTM C18 guard column (3.9×20 mm i.d., 10 µm, Waters Co.). The mobile phase consisted of acetonitrile: 0.01 M ammonium formate (pH 2.7, adjusted with phosphoric acid) (56:44, v/v). The flow-rate of the mobile phase was maintained at 1.0 ml/min. Chromatography was performed at 25°C and regulated by an HPLC column temperature controller. The UV detector was operated at a wavelength of 244 nm. Plasma calibration curves were prepared and assayed in triplicate on three different days to evaluate linearity,

precision, accuracy, recovery, limit of quantitation (LOQ), limit of detection (LOD), selectivity and stability.

2.5 Determination of blood glucose concentrations

Blood samples were collected at 0, 1, 3, 6, and 10 h after oral and intravenous repaglinide administration. Blood glucose concentrations were measured immediately using the Onetouch® UltraTM Blood Glucose Monitoring System and the glucose assay kit from BioAssay Systems (QuantiChromTM Glucose Assay Kit) as described by Yoon and Mekalanos [18].

2.6 CYP inhibition assay

The inhibition assays of human CYP3A4 enzyme activity were performed in a multiwell plate using the CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously [19]. Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrate [7-Benzyloxy-4-(trifluoromethyl) couamrin, 7-BFC for CYP3A4] was incubated (2 μM of both CYP isoforms and 50 mM of 7-BFC were used in the incubation mixture as described in the manual of CYP inhibition assay kit) with or without aspirin in enzyme/substrate buffer consisting of 1 pmol of P450 enzyme and a 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 $MgCl_2$) in potassium phosphate buffer (pH 7.4). Reactions were terminated by adding stop solution after 45-min incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. A positive

control (1 μM ketoconazole) was run on the same plate and produced 99% inhibition. All experiments were performed in duplicate, and results are expressed as the percent of inhibition.

2.7 Rhodamine-123 retention assay

The procedures used for the Rho-123 retention assay were similar to a previously reported method [20]. The P-gp overexpressed multidrug resistant human breast carcinoma cell line (MCF-7/ADR cells) were seeded in 24-well plates at a seeding density of 10^5 cells and preincubated with aspirin and verapamil for 30 min. At 80% confluency, the cells were incubated in fetal bovine serum free Dulbecco's modified Eagle's medium for 18 h. The culture medium was changed to Hanks' balanced salt solution and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20 μM rhodamine-123 in the presence or absence of aspirin (10, 30 and 100 μM) for 90 min, the medium was completely removed. The cells were then washed three times with ice-cold phosphate buffer (pH 7.0) and lysed in lysis buffer. Rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and are presented as the ratio to control.

2.8 Pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using Thermo Kinetica Software Version 5.0 (Thermo Fisher Scientific Inc., Miami, OK, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of repaglinide

concentration during the elimination phase. The terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak plasma concentration (C_{max}) and time to reach peak plasma concentration (T_{max}) of repaglinide in plasma were obtained by visual inspection of the data from the concentration–time curve. The area under the plasma concentration-time curve from 0 h to the time (AUC_{0-t}) of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC from zero to infinity ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. Total body clearance (CL/F) was calculated by:

$$CL/F = \frac{Dose}{AUC}$$

The mean residence time (MRT) was calculated by dividing the first moment of AUC ($AUMC_{0-\infty}$) by $AUC_{0-\infty}$. The apparent volume of distribution at steady state (Vd_{ss}) was estimated by the product of $MRT_{i.v.}$ and CL_t after i.v. dosing. The absolute bioavailability ($A.B.$) was calculated by:

$$AB\% = \frac{AUC_{oral}}{AUC_{i.v.}} \times \frac{Dose_{i.v.}}{Dose_{oral}} \times 100\%$$

The relative bioavailability ($R.B.$) was calculated by:

$$RB\% = \frac{AUC_{with\ aspirin}}{AUC_{control}} \times 100\%$$

2.9 Statistical analysis

Statistical analysis was conducted using one-way ANOVA followed by a posteriori testing with the Dunnett correction. Differences were considered significant at $P < 0.05$. All mean values are presented with their standard deviation (Mean ± SD).

3. Results

3.1 Chromatographic separation

Figure 9 illustrates chromatograms of the blank rat plasma samples (A), the plasma spiked with repaglinide, indomethacin (internal standard, IS) (B), and aspirin (C). The retention times of repaglinide and indomethacin (IS) were 6.8 and 8.5 min, respectively. The overall run time lasted 10 min. Aspirin did not interfere with the detection of repaglinide and indomethacin (IS) in this method.

3.2 Validation characteristics of analysis

Figure 10 shows the calibration curve of repaglinide constructed by plotting the ratio of the peak area of repaglinide to that of indomethacin as a function of the plasma repaglinide concentrations (50, 100, 200, 500, 1000, 2000 and 5000 ng/ml). There was excellent linearity over the range of 50–5000 ng/ml with a mean correlation coefficient of 0.999. The typical equation describing the calibration curve in rat plasma was $y = 0.0031\ x - 0.0052$, where y is the peak area ratio of repaglinide to indomethacin and x is the concentration of repaglinide. The recovery of repaglinide in rat plasma was 102.2%. The LOD and LOQ for repaglinide in rat plasma were 20 ng/ml. The intra- and inter-day coefficients for variation of repaglinide were below 12.1 and 11.5%, respectively.

3.3 CYP inhibition assay

The inhibitory effect of ketoconazole and aspirin on CYP3A4 enzyme activity is shown

in Figure 11. Ketoconazole and aspirin inhibited CYP3A4 enzyme activity in a concentration-dependent manner. Ketoconazole and aspirin inhibited CYP3A4 enzyme activity with IC_{50} values of 0.03 and 15.0 μM, respectively.

3.4 Rhodamine-123 retention assay

The effect of aspirin on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells is shown in Figure 12. Accumulation of rhodamine-123 was reduced in MCF-7/ADR cells overexpressing P-gp compared to that in MCF-7 cells lacking P-gp. The relative cellular uptake of rhodamine-123 was comparable at the concentration range of 10-100 μM aspirin. This result suggests that aspirin did not inhibit P-gp activity.

3.5 Determination of blood glucose concentrations

Repaglinide pharmacodynamics was characterized by determining blood glucose levels. Mean blood glucose–time profiles in rats after oral (0.5 mg/kg) and intravenous (0.2 mg/kg) administration of repaglinide in the presence or absence of aspirin (5.0 and 20.0 mg/kg) (n=6, each) are shown in Figure 13 and 14. Compared to the control group, aspirin significantly decreased the blood glucose levels after oral administration of repaglinide, revealing a significant hypoglycemic effect (Figure 13); however, there was no significant difference after intravenous administration of repaglinide (Figure 14).

3.6 Effect of aspirin on the pharmacokinetics of oral repaglinide

Mean arterial plasma concentration-time profiles of repaglinide following oral administration of repaglinide (0.5 mg/kg) to rats in the presence or absence of aspirin (5.0

and 20.0 mg/kg) are shown in Figure 15; the corresponding pharmacokinetic parameters are shown in Table 3. Aspirin significantly altered the pharmacokinetic parameters of repaglinide. Compared to the control group (given oral repaglinide alone), aspirin significantly increased the area under the plasma concentration-time curve ($AUC_{0-\infty}$, $P < 0.05$ at 20.0 mg/kg) and the peak plasma concentration (C_{max}, $P < 0.05$ at 20.0 mg/kg) of repaglinide by 36.6% and 18.8%, respectively. The total body clearance (*CL/F*) was significantly decreased (20.0 mg/kg, $P < 0.05$) by aspirin. Aspirin increased the absolute bioavailability (*A.B.*) of repaglinide by 36.9% (20.0 mg/kg, $P < 0.05$) compared to the oral control group, and the relative bioavailability (*R.B.*) of repaglinide by 1.13- to 1.37-fold. There were no significant differences in the time to reach peak plasma concentration (T_{max}), terminal half-life ($t_{1/2}$) of repaglinide in the presence of aspirin.

3.7 Effect of aspirin on the pharmacokinetics of intravenous repaglinide

Mean plasma concentration-time profiles of repaglinide after the intravenous administration of repaglinide (0.2 mg/kg) to rats in the presence or absence of aspirin (5.0 and 20.0 mg/kg) are shown in Figure 16, correlated pharmacokinetic parameters are shown in Table 4. The $AUC_{0-\infty}$ of repaglinide was increased, but was not statistically significant compared to that in the control. The CL_t and Vd_{ss} values of repaglinide tended to decrease, but this trend was not statistically significant. The $t_{1/2}$ of repaglinide was also prolonged, but this increase was not significant. In contrast to those of oral repaglinide, the pharmacokinetics of intravenous repaglinide were not affected by the concurrent use of aspirin. Accordingly, the enhanced oral bioavailability in the presence of aspirin, while there was no significant change in the pharmacokinetics of intravenous repaglinide, may be

mainly due to inhibition of the CYP3A isoforms-mediated metabolism of repaglinide in the small intestine and/or in liver by aspirin rather than by renal elimination of repaglinide.

4. Discussion

Type 2 diabetes mellitus is a common health problem associated with cardiovascular disease, with an increasing incidence worldwide [21]. Aspirin, an antiplatalet agent, is one of the most widely employed drugs in the prevention of cardiovascular disease [22]. As so-called secondary prevention, it is effective for the prevention of cardiovascular events in patients with a history of vascular disease. In the general population with no history of previous myocardial infarction or stroke, aspirin also seems to be useful for primary prevention of cardiovascular events, although the absolute benefits are smaller than those seen in patients with previous cardiovascular disease [23]. Mazzone reported that aspirin has been recommended for primary prevention in diabetic patients at risk of recurrent cardiovascular risk [16]. Clinically, repaglinide and aspirin, an antiplatelet agent, could be prescribed for prevention or treatment of diabetes and cardiovascular disease as a complication of diabetes. Considering that the drugs used in combination therapy often share the same metabolic pathways or cellular transport pathways, there is a high potential for pharmacokinetic and pharmacodynamic drug interactions between repaglinide and aspirin. Consequently, this study was designed to investigate the effect of aspirin on CYP3A4 enzyme activity, P-gp activity and the pharmacokinetics or pharmacodynamics of repaglinide after oral and intravenous administration of repaglinide in rats.

The cytochrome P450 superfamily is a large and diverse group of enzymes. Most CYP enzymes function to catalyze the oxidation of organic substances. The substrates of CYP enzymes include metabolic intermediates such as lipids and steroidal hormones, as well as xenobiotic substances such as drugs and other toxic chemicals. CYPs are the major enzymes

involved in drug metabolism and bioactivation, accounting for about 75% of the total number of different metabolic reactions [24].

Many drugs may increase or decrease the activity of various CYP isozymes either by inducing the biosynthesis of an isozyme (enzyme induction) or by directly inhibiting the activity of the CYP (enzyme inhibition). This is a major source of adverse drug interactions, since changes in CYP enzyme activity may affect the metabolism and clearance of various drugs. For example, if one drug inhibits the CYP-mediated metabolism of another drug, the second drug may accumulate within the body to toxic levels. Hence, these drug interactions may necessitate dosage adjustments or choosing drugs that do not interact with the CYP system. Such drug interactions are especially important to take into account when using drugs of vital importance to the patient, drugs with important side-effects and drugs with small therapeutic windows, but any drug may be subject to an altered plasma concentration due to altered drug metabolism.

One of the principal cytochrome P450 isoforms involved in repaglinide metabolism in human liver microsomes is CYP3A4. CYP3A4 is the most abundant isoform of cytochrome P450 in the human liver [25] and is responsible for the metabolism of many different drugs [26]. Levels of CYP3A4 vary substantially between individuals; these variations may be related to induction of the isoform by various drugs and may partially account for the observed inter-individual variations in repaglinide metabolism [27]. As CYP3A9 expressed in rat corresponds to the ortholog of CYP3A4 in human [28], rat CYP3A2 is similar to human CYP3A4 [29, 30]. Human 3A4 and rat 3A1 have 73% of protein homology [31]. Rats were selected as an animal model in this study to evaluate the potential pharmacokinetic interactions mediated by CYP3A4, although there should be

some difference in enzyme activity between rat and human [32].

Considering that repaglinide is a substrate of both CYP3A isoforms and P-gp [14, 16], the effects of aspirin on the CYP enzyme activity and the cell-based P-gp activity were evaluated. As shown in Figure 1, aspirin inhibited CYP3A4 activity with IC_{50} value of 15.0 μM. However, the relative cellular uptake of rhodamine-123 was comparable (Figure 2). This suggested that aspirin could not inhibit P-gp activity. The inhibitory effect of aspirin against CYP3A isoforms-mediated metabolism was confirmed by the employment of recombinant CYP enzymes. Poor solubility and first-pass metabolism in the liver and epithelial cells of the small intestine result in low bioavailability of repaglinide (56%) [16], and CYP3A inhibitors might alter the bioavailability and pharmacokinetics of repaglinide. The inhibitory effect of aspirin against CYP3A isoforms-mediated metabolism was confirmed through the use of recombinant CYP3A enzyme. Aspirin is rapidly hydrolyzed into salicylic acid in intestine and liver after oral administration, but the plasma concentration of aspirin after oral administration is over 20 μg/ml in human and rat [33, 34]. This result supports that its IC_{50} for CYP3A4 inhibition is physiologically relevant and that aspirin might therefore affect the bioavailability and pharmacokinetics of repaglinide through CYP3A4 inhibition.

In this study, aspirin significantly decreased the blood glucose levels compared to the oral control group and increased the $AUC_{0-\infty}$ by 36.6% and the *R.B.* of repaglinide by 1.13- to 1.37-fold. Aspirin considerably increased the C_{max} of repaglinide, it seems that aspirin inhibited the CYP3A isoforms-mediated biotransformation of repaglinide mainly during first-pass metabolism. CYP3A isoforms is present in considerable quantities in the small-intestine mucosa [35, 36], and the intestine CYP3A isoforms has been shown to play a

major role in drug interactions with CYP3A isoforms inhibitors. These results are consistent with a report by Niemi *et al*. [37] showing that clarithromycin, a CYP3A4 inhibitor, significantly increased the $AUC_{0-\infty}$ and C_{max} of repaglinide and enhanced the repaglinide blood glucose-lowering effect, despite that no statistically significant differences were found in the blood concentrations between control group and clarithromycin. Niemi *et al*. [38] reported that gemfibrozil significantly increased the $AUC_{0-\infty}$ of repaglinide 8.1-fold and enhanced the blood glucose-lowering effect of repaglinide. This result is also consistent with our study.

After intravenous administration of repaglinide with aspirin, the $AUC_{0-\infty}$ of repaglinide was increased, but this effect was not significant. The CL_t and Vd_{ss} values of repaglinide tended to decrease, but these tends were not statistically significant. This suggests that the effects of oral aspirin on the inhibition of hepatic metabolism of repaglinide via CYP3A isoforms were almost negligible. In contrast to those of oral repaglinide, the pharmacokinetics of intravenous repaglinide were not affected by the concurrent use of aspirin. Accordingly, while there was no significant change in the pharmacokinetics of intravenous repaglinide, the enhanced oral bioavailability may be mainly due to inhibition of the CYP3A isoforms-mediated metabolism of repaglinide in the small intestine and/or liver by aspirin rather than renal elimination of repaglinide. Although there are potential adverse effects, this interaction may provide a therapeutic benefit whereby it enhances bioavailability, allowing a reduction in the dose administered. Since the present study has raised the awareness of potential drug interactions by concomitant use of aspirin with repaglinide, the clinical significance of this finding needs to be further evaluated in clinical studies.

5. Conclusion

Aspirin, an antiplatalet agent, significantly enhanced the oral bioavailability of repaglinide. The enhanced bioavailability of repaglinide may be attributable to the inhibition of the CYP3A isoforms-mediated metabolism in the small intestine and/or liver and to reduction of total body clearance rather than both to inhibition of P-gp in the small intestine and to reduction of renal elimination of repaglinide by aspirin.

The increase in the oral bioavailability of repaglinide should be taken into consideration of potential drug interactions when coadministering repaglinide and aspirin. Furthermore, the blood glucose concentrations should be carefully monitored during their coadministration.

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Table 3. Pharmacokinetic parameters of repaglinide after the oral administration of repaglinide (0.5 mg/kg) to rats in the presence or absence of aspirin (5.0 and 20.0 mg/kg) (n=6, each).

Parameter	Repaglinide (Control)	Repaglinide + Aspirin	
		5.0 mg/kg	20.0 mg/kg
$AUC_{0-\infty}$ (ng·h/ml)	545.4 ± 98.2	616.3 ± 117.1	744.8 ± 126.6*
C_{max} (ng/ml)	185.2 ± 20.3	194.1 ± 28.9	220.0 ± 13.8*
T_{max} (h)	0.67 ± 0.13	0.71 ± 0.10	0.79 ± 0.10
$t_{1/2}$ (h)	2.5 ± 0.5	2.7 ± 0.5	2.9 ± 0.7
CL/F (ml/min/kg)	15.3 ± 2.8	13.5 ± 2.6	11.3 ± 1.9*
A.B. (%)	33.9 ± 6.2	38.4 ± 7.3	46.4 ± 7.9*
R.B. (%)	100	113	137

* $P < 0.05$, significant difference 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; CL/F: total body clearance; *A.B.*: absolute bioavailability; *R.B.*: relative bioavailability.

Table 4. Pharmacokinetic parameters of repaglinide after the intravenous administration of repaglinide (0.2 mg/kg) to rats in the presence or absence of aspirin (5.0 and 20.0 mg/kg) (n=6, each).

Parameter	Repaglinide (Control)	Repaglinide + Aspirin	
		5.0 mg/kg	20.0 mg/kg
$AUC_{0-\infty}$ (ng·h/ml)	642.7 ± 115.7	691.1 ± 121.3	745.4 ± 139.7
$t_{1/2}$ (h)	2.6 ± 0.5	2.7 ± 0.6	2.8 ± 0.7
CL_t (ml/min/kg)	5.2 ± 1.3	4.8 ± 1.1	4.5 ± 1.0
Vd_{ss} (ml/kg)	13.0 ± 2.4	12.6 ± 2.3	12.4 ± 2.1

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; $t_{1/2}$: terminal half-life; CL_t: total body clearance; Vd_{ss}: volume of distribution at steady state.

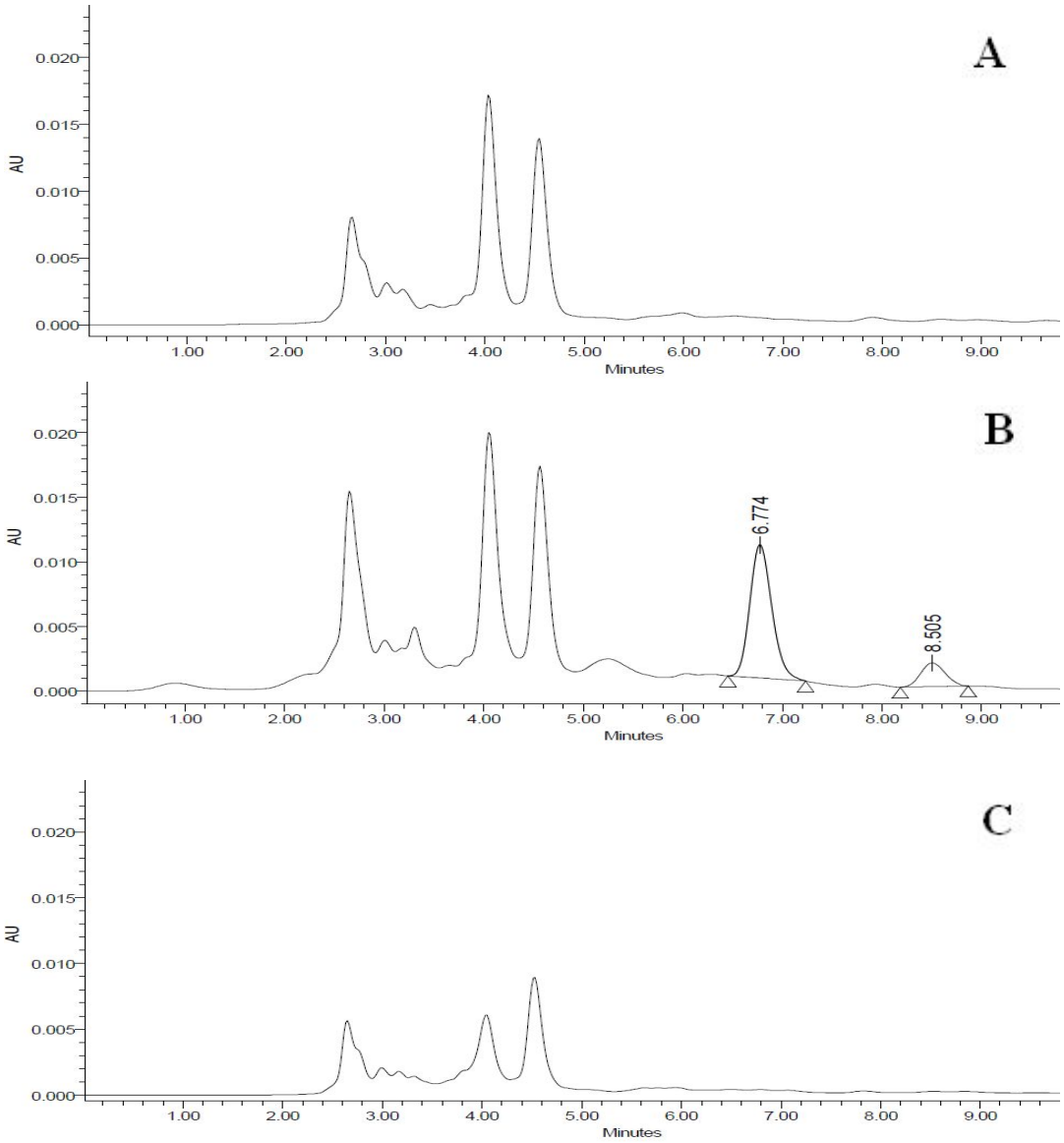


Figure 9. HPLC chromatograms of the blank rat plasma (A), the plasma spiked with repaglinide (6.8 min), indomethacin (internal standard; 8.5 min) (B), and aspirin in the rat plasma (C).

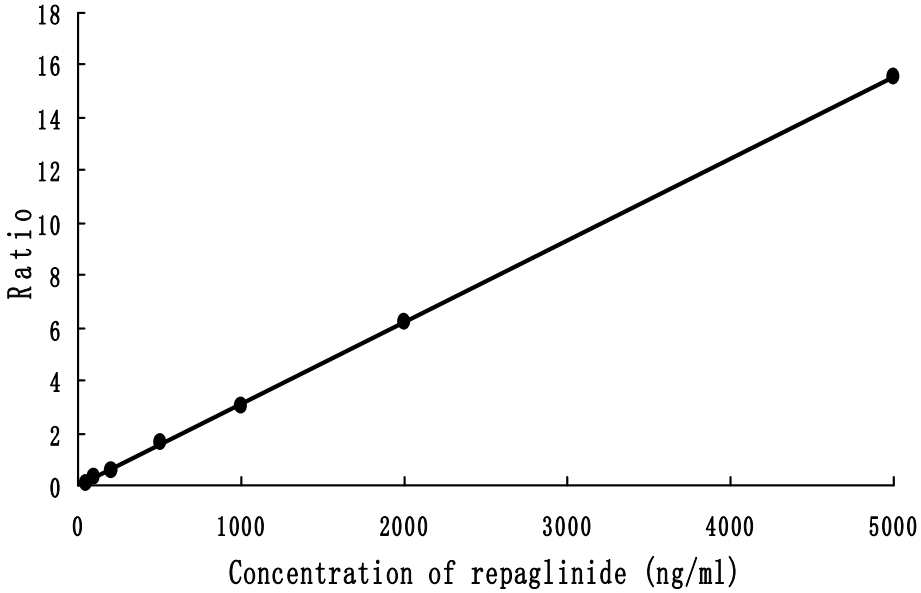
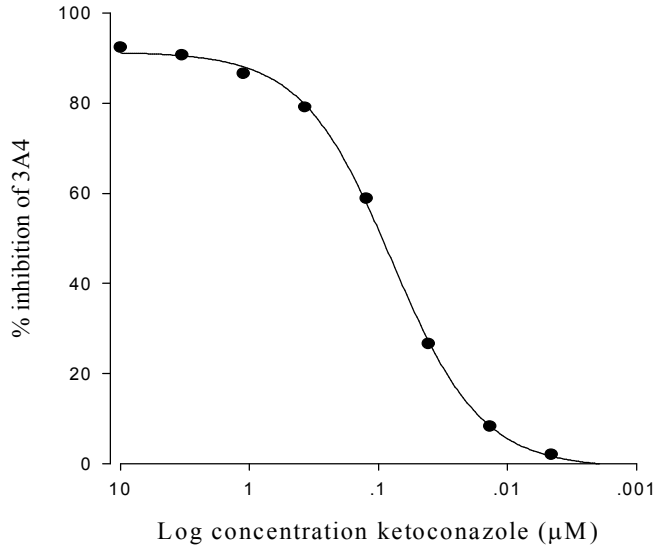


Figure 10. A calibration curve of repaglinide was linear in the concentration range of 50–5000 ng/ml in rat plasma. The typical equation describing the calibration curve in rat plasma was $y = 0.0031\ x - 0.0052$, where "y" is the peak area ratio of repaglinide to indomethacin and "x" is the concentration of repaglinide.



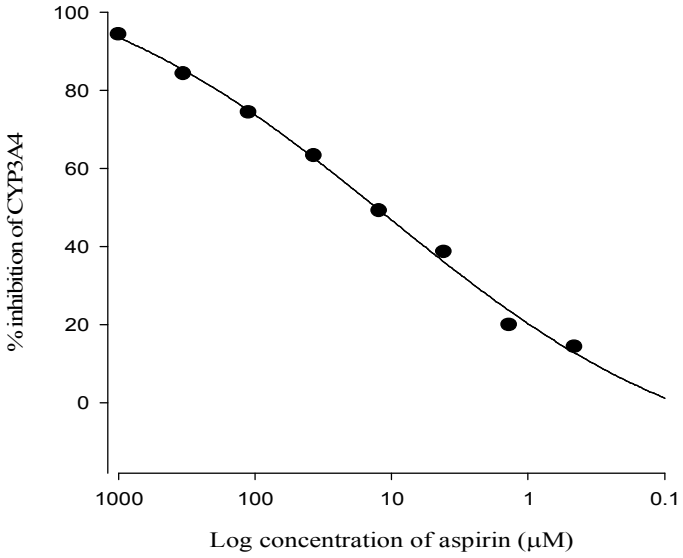


Figure 11. Inhibitory effects of ketoconazole and aspirin on CYP3A4 activity. The experiment was done in duplicate, and the result is expressed as the percent of inhibition.

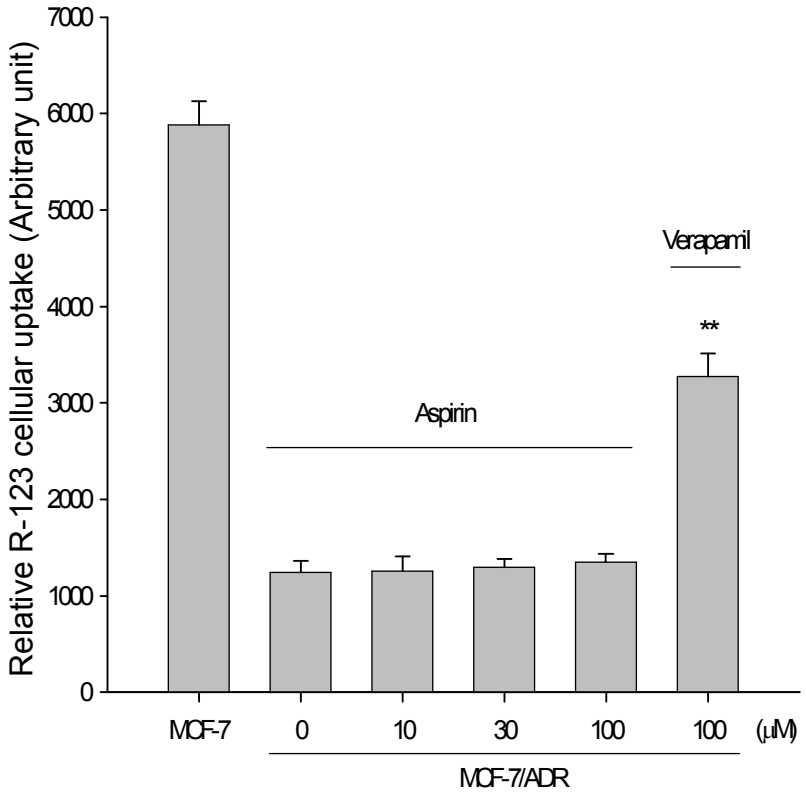


Figure 12. Rhodamine-123 retention. MCF-7/ADR cells were preincubated with aspirin for 30 min after incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min. Data represents mean ± SD of 6 separate samples (significant versus the control MCF-7 cells, ** $P < 0.01$).

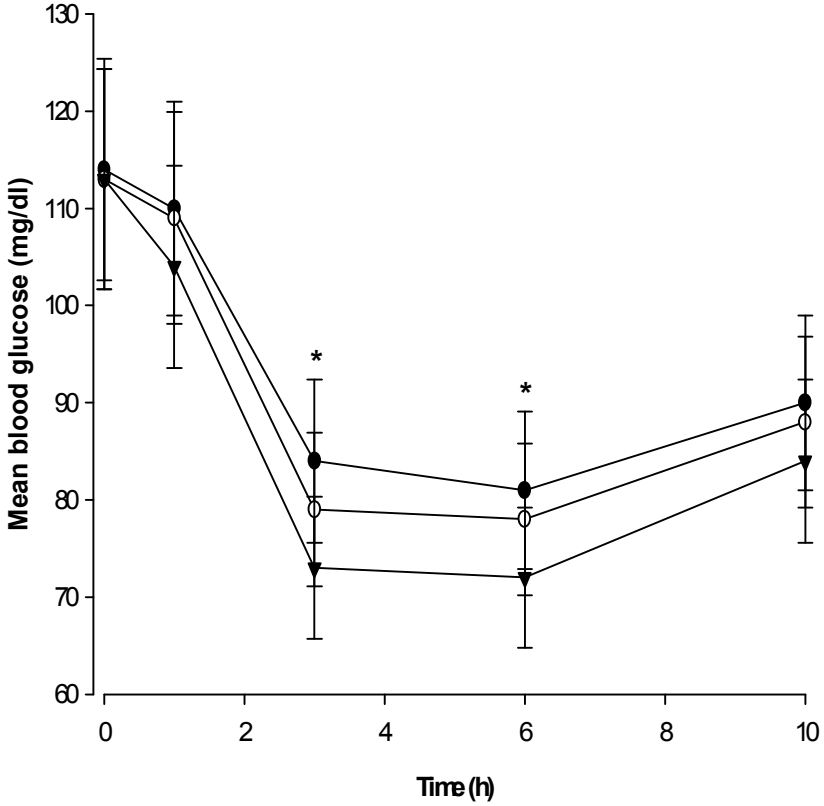


Figure 13. Mean blood glucose–time profiles in rats after oral (0.5 mg/kg) administration of repaglinide in the presence or absence of aspirin (5.0 and 20.0 mg/kg) (*n*=6, each). Bars represent the standard deviation; Oral administration of 0.5 mg/kg repaglinide (control) (solid circles ●), with 5.0 mg/kg aspirin (open circles ○) and with 20.0 mg/kg aspirin (solid triangles ▼) (* $P < 0.05$).

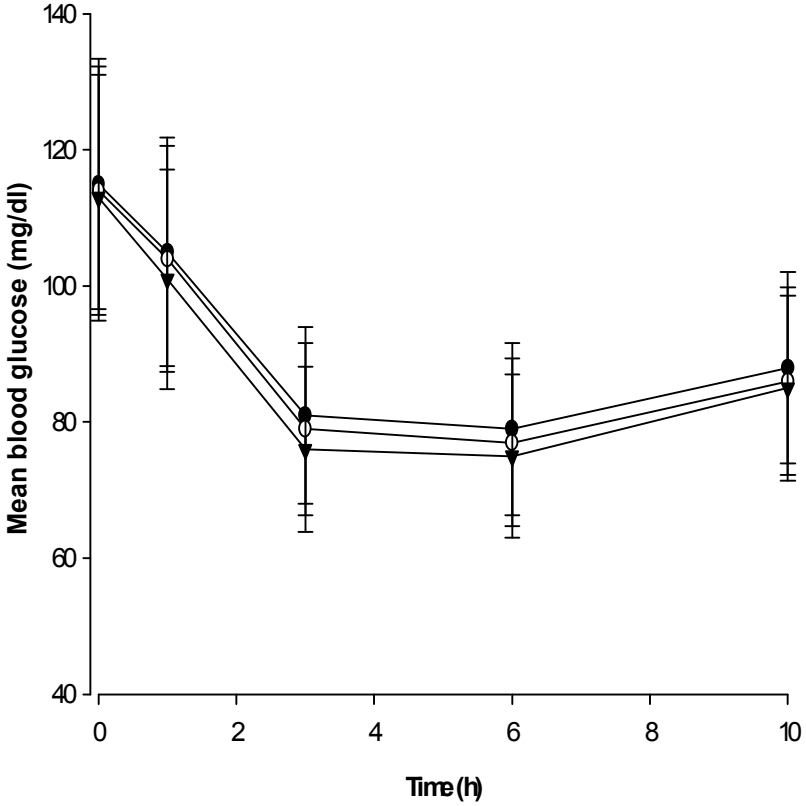


Figure 14. Mean blood glucose–time profiles in rats after intravenous (0.2 mg/kg) administration of repaglinide in the presence or absence of aspirin (5.0 and 20.0 mg/kg) (*n*=6, each). Bars represent the standard deviation; Intravenous administration of 0.2 mg/kg repaglinide (control) (solid circles ●), with 5.0 mg/kg aspirin (open circles ○) and with 20.0 mg/kg aspirin (solid triangles ▼).

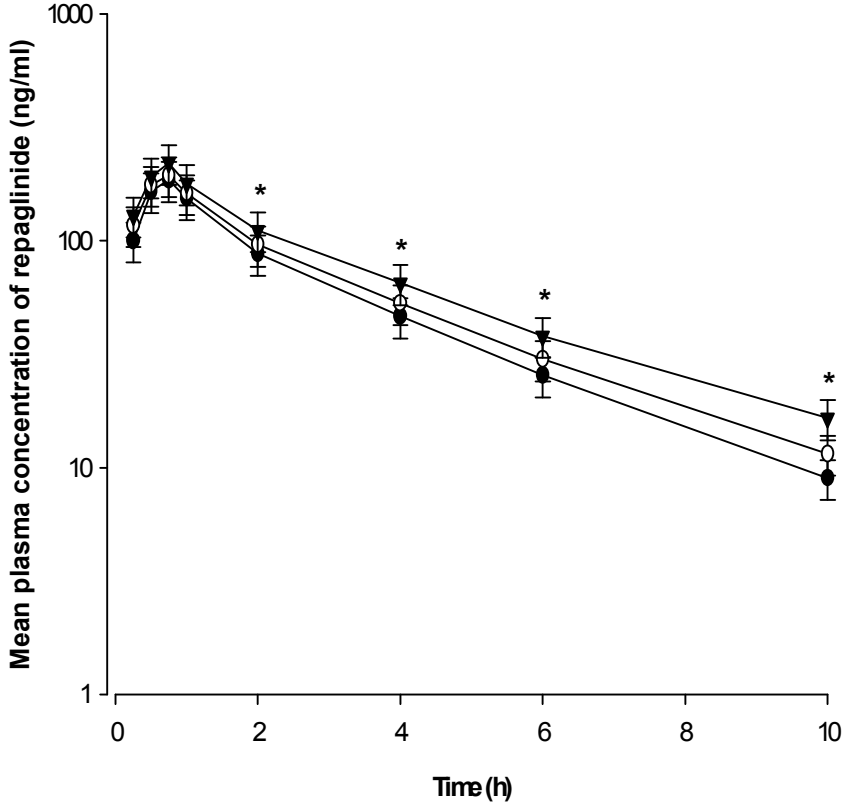


Figure 15. Mean plasma concentration–time profiles of repaglinide after oral (0.5 mg/kg) administration of repaglinide to rats in the presence or absence of aspirin (5.0 and 20.0 mg/kg) (n=6, each). Bars represent the standard deviation; Oral administration of 0.5 mg/kg repaglinide (control) (solid circles ●), with 5.0 mg/kg aspirin (open circles ○) and with 20.0 mg/kg aspirin (solid triangles ▼) (* $P < 0.05$).

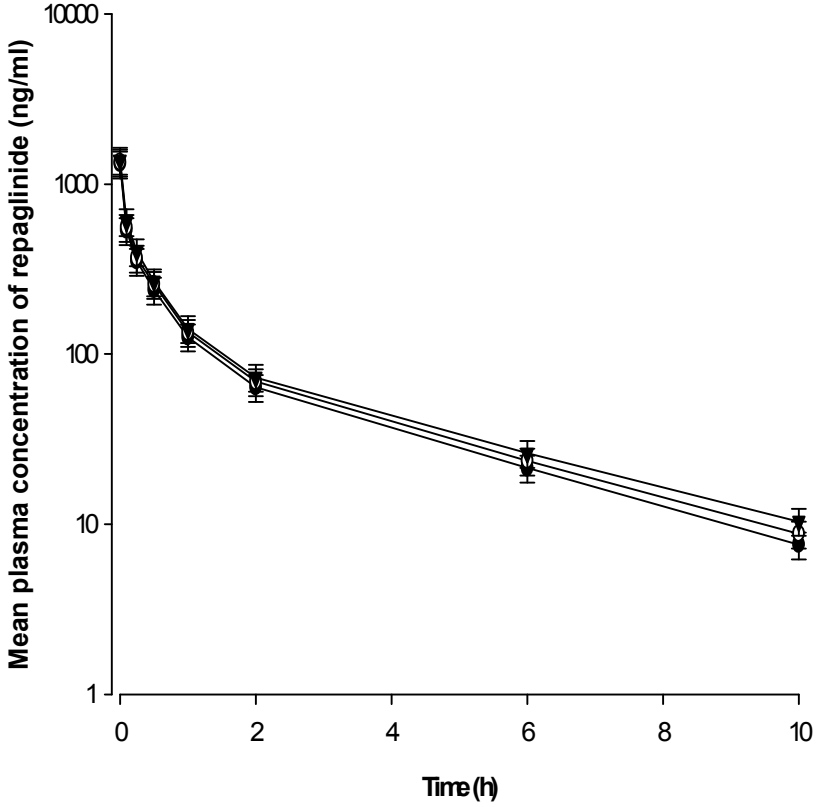


Figure 16. Mean plasma concentration–time profiles of repaglinide after intravenous (0.2 mg/kg) administration of repaglinide to rats in the presence or absence of aspirin (5.0 and 20.0 mg/kg) (*n*=6, each). Bars represent the standard deviation; Intravenous administration of 0.2 mg/kg repaglinide (control) (solid circles ●), with 5.0 mg/kg aspirin (open circles ○) and with 20.0 mg/kg aspirin (solid triangles ▼).

Abstract

Repaglinide and aspirin interact with the same cytochrome P450 (CYP)3A isoforms and clinically repaglinide and aspirin can be prescribed for prevention or treatment of diabetes and cardiovascular disease as complications of diabetes. The purpose of this study was to investigate the effects of aspirin, an antiplatalet agent, on the bioavailability and pharmacokinetics of repaglinide in rats.

The effects of aspirin on P-glycoprotein (P-gp) and CYP3A4 enzyme activity was evaluated. The pharmacokinetic parameters of repaglinide and blood glucose concentrations were also determined in rats after oral (0.5 mg/kg) and intravenous (0.2 mg/kg) administration of repaglinide to rats in the presence and absence of aspirin (5.0 and 20.0 mg/kg).

Aspirin inhibited CYP3A4 enzyme activity with IC_{50} value of 15.0 μM and the relative cellular uptake of rhodamine-123 was comparable. Compared to the oral control group, aspirin significantly increased the $AUC_{0-\infty}$ and the C_{max} of repaglinide by 36.6% and 18.8%, respectively. The *CL/F* was significantly decreased by aspirin. Aspirin also increased the absolute bioavailability (*A.B.*) of repaglinide by 36.9% compared to the oral control group. Moreover, the relative bioavailability (*R.B.*) of repaglinide was 1.13- to 1.37-fold greater than that of the control group. The blood glucose concentrations were significantly different compared to the oral control groups.

Aspirin enhanced the oral bioavailability of repaglinide, and this may be attributable to the inhibition of the CYP3A isoforms-mediated metabolism in the small intestine and/or liver and to reduction of total body clearance rather than to inhibition of P-gp in the small

intestine and reduction of renal elimination of repaglinide by aspirin. The increase in the oral bioavailability of repaglinide should be taken into consideration of potential drug interactions when coadministering repaglinide and aspirin. Furthermore, the blood glucose concentrations should be carefully monitored during their coadministration.

Key words: Repaglinide · Pharmacokinetics · Pharmacodynamics · Aspirin · CYP3A isoforms · blood glucose · Rats

ABSTRACT

Effects of Efonidipine and Aspirin on the Pharmacokinetics and Pharmacodynamics of Repaglinide in Rats

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Efonidipine is a novel dihydropyridine calcium antagonist and is a powerful vasodilator drug. Aspirin is a salicylate drug and recently aspirin has been recommended for primary prevention for diabetic patients at risk of recurrent cardiovascular risk. In clinically, repaglinide and efonidipine or aspirin can be prescribed for treatment of cardiovascular disease as complications of diabetes. On the other hand, repaglinide and efonidipine or aspirin interact with the same CYP3A isoforms and P-gp. Therefore, the present study aims to investigate the effect of efonidipine or aspirin on the pharmacokinetics and pharmacodynamics of repaglinide after oral and intravenous administration in rats.

The pharmacokinetic parameters of repaglinide and blood glucose concentrations were determined after oral and intravenous administration of repaglinide (0.5 or 0.2 mg/kg) to rats in the presence and absence of efonidipine (1.0 and 3.0 mg/kg) or aspirin (5.0 and 20.0 mg/kg). The effects of efonidipine or aspirin on the CYP3A4 activity as well as P-gp activity were also evaluated. Efonidipine and aspirin significantly inhibited CYP3A4

enzyme. In addition, efonidipine significantly reduced rhodamine-123 efflux via P-gp in MCF-7/ADR cell overexpressing P-gp.

Compared to the control (repaglinide alone), efonidipine or aspirin significantly altered the pharmacokinetic parameters of repaglinide. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak plasma concentration (C_{max}) of repaglinide were significantly increased in the presence of efonidipine or aspirin, respectively. The total body clearance (*CL/F*) was significantly decreased by efonidipine or aspirin, respectively. Consequently, the absolute bioavailability (*A.B.*) of repaglinide in the presence of efonidipine or aspirin were significantly higher than that of the control group, respectively. Compared to the intravenous control, efonidipine significantly increased the plasma concentration-time curve ($AUC_{0-\infty}$) of intravenously administered repaglinide. However, pharmacokinetics parameters of intravenous repaglinide were not affected by aspirin. The blood glucose concentrations have significant differences in the presence and absence of efonidipine or aspirin compared to the oral control groups.

In conclusion, the enhanced oral bioavailability of repaglinide by efonidipine or aspirin may result from decreased P-gp-mediated efflux in small intestine and inhibition of CYP 3A isoforms-mediated metabolism in small intestine and/or in the liver and reduction of total body clearance of repaglinide.

Based on the results, if the drug interactions are confirmed in the diabetes patients with cardiovascular diseases, the dosage of repaglinid should be readjusted and blood glucose concentrations should be carefully monitored when repaglinide is used concomitantly with efonidipine or aspirin.

Key words: Repaglinide · Pharmacokinetics · Pharmacodynamics · Efonidipine · Aspirin · CYP3A isoforms · P-gp · Rats

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