2010 년 2 월
석사학위논문

Pharmacokinetic Interaction between Losartan and Ticlopidine in Rats

조선대학교 대학원

약학과

김 형 기

Pharmacokinetic Interaction between Losartan and Ticlopidine in Rats

흰쥐에서 로살탄과 티크로피딘과의 상호작용

2010 년 2 월 25 일

조선대학교 대학원

약학과

김 형 기

Pharmacokinetic Interaction between Losartan and Ticlopidine in Rats

지도교수 최 준 식

이 논문을 약학석사학위신청 논문으로 제출함.

2009 년 10 월

조선대학교 대학원

약학과

김 형 기

김형기의 석사학위논문을 인준함

위원장 조선대학교 교수 한효경 인

위 원 조선대학교 교수 강건욱 인

위 원 조선대학교 교수 최준식 인

2009 년 11 월

조선대학교 대학원

CONTENTS

LIST OF FIGURES

LIST OF TABLES

Abstract

Pharmacokinetic Interaction between Losartan and Ticlopidine in Rats

Hyung-ki Kim

Advisor: Prof. Jun-Shik Choi, Ph.D.

Department of Pharmacy,

Graduate School Chosun University

The present study was to investigate the effect of ticlopidine and its metabolited(EXP-3174), antiplatelet drug, on the pharmacokinetics of losartan, substrates of CYP3A4, 2C9 and P-gp, in rats. Pharmacokinetic parameters of losartan and EXP-3174 in rats were determined after an oral administration of losartan (9 mg/kg) in the presence or absence of ticlopidine (4 and 10 mg/kg). The pharmacokinetic parameters of losartan were significantly altered by the presence of ticlopidine compared with the control group (given losartan alone). Presence of ticlopidine significantly ($p < 0.05$ 4mg/kg; $p < 0.01$, 10 mg/kg) increased the area under the plasma concentration–time curve (AUC) of losartan by 30.7–73.1% and peak plasma concentration (C_{max}) of losartan by 17.3–40.0%, while total plasma clearance (CL/F) of losartan was decreased by 23.5–42.3%. However there were no significant changes in the volume of distribution(Vdss), T_{max} and terminal half life($t_{1/2}$) of losartan in the presence of ticlopidine. Consequently, the absolute bioavailability (AB) of losartan in the presence of ticlopidine was 20.6–27.3%,

which was enhanced significantly ($p < 0.05$ 4mg/kg; $p < 0.01$, 10 mg/kg) compared with the control group (15.8%). The relative bioavailability (RB) of losartan increased by 1.30 to 1.72 fold in the presence of ticlopidine. Presence of ticlopidine (10 mg/kg) significantly increased the AUC (41.4%) of EXP-3174 compared with the control group. The metabolite-parent AUC ratio (MR) was significantly ($P<0.05$) decreased by 10.1-18.2% in the presence of ticlopidine (4 and 10mg/kg) compared to the control group. However there were no significant changes in the volume of distribution(Vdss), T_{max} and terminal half life($t_{1/2}$) of EXP-3174 in the presence of ticlopidine. Ticlopidine significantly enhanced bioavailabiliy of losartan in rats. The increased bioavailability was due to inhibition of the CYP3A4 and CYP2C9-mediated metabolism of losartan in the small intestine or in the liver, and further, due to decreased total body clearance(CL/F) of losartan. However, P-gp activating was not altered in ticlopidine-treated MCF-7/ADR cells. In conclusion, the presence of ticlopidine significantly enhanced the bioavailability of losartan. So, concurrent use of ticlopidine with losartan should require close monitoring for potential drug interactions in clinics.

Key words**:** Losartan, EXP-3174, Ticlopidine, Pharmacokinetics, CYP, P-gp, Rat.

국 문 초 록

흰쥐에서 로살탄과 티크로피딘과의 상호작용

김 형 기

지 도 교 수: 최 준 식

조선대학교대학원 약학과

로살탄(losartan)은 CYP3A4, CYP2C9, P-gp의 기질이며 순환기계 약물로서 안지오텐신 수용체를 차단하여 고혈압치료에 널리 사용된다. 티크로피딘(ticlopidine)은 항혈소판작용을 하여 주로 심장질환 특히 관상동맥 질환인 협심증 예방 및 치료 처방으로 빈용되고 있다. 따라서 본 실험에서는 흰쥐에게 티크로피딘 (4mg/kg 및 10 mg/kg)과 로살탄을 경구 (9 mg/kg) 병용투여하였을 때 로살탄 및 그 활성대사체인 EXP-3174의 약물동태학적 파라미터를 연구검토하였다.

티크로피딘과 동시투여하였을 때 로살탄의 약물동태학적 파라미터는 유의성 있게 변화 하였다. 혈장농도곡선하면적 (AUC)는 티크로피딘 병용투여군에서 대조군에 비해 유의성 (4mg/kg, $P<0.05$; 10mg/kg, $P<0.01$)있게 증가하였으며 최고혈중농도 (C_{max})도 유의성 ($p<0.05$) 있게 증가하였다. 토탈클리어런스(CL/F)는 유의성(4mg/kg, $P<0.05$; 10mg/kg,

P<0.05)있게 감소하였다. 절대적생체이용율(AB)은 대조군에 비해 유의성 (4mg/kg, P<0.05; 10mg/kg, P<0.01) 있게 증가되었다. 그 결과로 상대적생체이용율(RB)이 1.30-1.72 배 증가되었다. 티크로피딘 병용투여군에서 소실반감기 ($t_{1/2}$)는 유의성(10mg/kg, P<0.05) 있게 증가하였고, 분포용적(Vdss)은 대조군에 비해 감소하였으나 유의성은 없었다. 티크로피딘 병용투여군에서 최고혈중농도 도달시간은 유의성이 없었다.

로살탄의 활성대사체인 EXP-3174에서 혈장농도곡선하면적 (AUC)는 티크로피딘 병용투여군에서 유의성 (10mg/kg, P<0.05) 있게 증가하였으며 토탈클리어런스(CL/F)는 유의성(10mg/kg, P<0.05) 있게 감소하였다. 최고혈중농도 (C_{max})도 유의성 (p<0.05) 있게 증가하였다. 분포용적(Vdss)은 대조군에 비해 감소하였으나 유의성이 없었고 소실반감기($t_{1/2}$)는 대조군에 비해 증가하였으나 유의성이 없었다. 또한 P-gp 발현이 높은 MCF-7/ADR 세포에서 티크로피딘의 P-gp에 대한 영향은 거의 없었다.

본 연구에서 고혈압 치료제인 로살탄과 항혈소판제인 티크로피딘을 동시투여하였을 때 경구투여 시킨 로살탄의 생체이용률의 증가는 CYP3A4, 2C9의 억제와 토탈클리어런스의 감소로 인한 것으로 사료된다. 본 연구결과를 토대로, 임상에서 티크로피딘과 로살탄의 병용투여시 로살탄의 용량을 조절하는 것이 바람직하다고 사료된다.

1. Introduction

Losartan potassium (DuP 753 or MK-954), an angiotensin II receptor antagonist, is the first of a new class of agents to be introduced for the treatment of hypertension [1, 2]. Two angiotensin receptor subtypes, angiotensin receptor-1 (AT_1) and angiotensin receptor-2 (AT_2), have been proposed on the basis of ligand-binding studies [3]. Studies confirm that losartan is an orally active, long-lasting selective antagonist of AT, receptors. Losartan is nearly completely absorbed and extensively metabolized to the active metabolite, EXP-3174 [4]. After oral losartan, about 5% of the dose is excreted unchanged in the urine and about 8% of the dose is excreted in the urine as EXP-3174. The remainder of the drug is excreted in urine and feces as inactive metabolites (oxidative metabolites or glucuronide conjugates). Rare (< 1%) individuals have been identified in whom the amount of losartan transformed to EXP-3174 appears to be less than 1% [5]. Andrea et al. suggested that losartan should be a substrate of both cytochrome P450 (CYP)3A and P-glycoprotein (P-gp) [6]. *In vitro* [7-9] and *in vivo* [10-12] studies demonstrated that losartan is metabolized by the CYP3A4. Considering that P-gp is co-localized with CYP3A4 in small intestine, P-gp and CYP3A4 may act synergistically for the presystemic drug metabolism and lead to the prolonged exposure of P-gp substrates to CYP3A4, resulting in the limited absorption of drugs [13-17].

Ticlopidine is a potent inhibitor of platelet aggregation induced by adenosine

diphosphate(ADP), whereas its ability to inhibit aggregation caused by thrombin, collagen, arachidonicacid, adrenalin, and platelet-activating factor is variable [18]. It has been tried in a variety of platelet-dependent disease states [19-21]. Several recent reviews recommend ticlopidine as a valuable alternative when patients cannot tolerate aspirin [22-27].

Clinically losartan and ticlopidine could be prescribed for treatment of cardiovascular disease therapy. However, little information is available on the *in vivo* effects of these drugs on the pharmacokinetics of the drug.

Given that the bioavailability of losartan is mainly affected by CYP3A4, CYP2C9 and P-gp during the first-pass metabolism, ticlopidine as a dual inhibitor of CYP3A4 and CYP2C9 may provide a therapeutic benefit to improve the pharmacokinetics of losartan in the combination therapy. Therefore, the present study aims to investigate the effect of ticlopidine on the pharmacokinetics of losartan and its active metabolite, EXP-3174, in rats.

2. Materials and methods

2.1. Chemicals

Losartan, EXP-3174 and L-158.809 (internal standard) were obtained from the Merck Co. (Darmstadt, Germany). Ticlopidine were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). Acetonitrile, methanol, tert-butylmethylether were purchased from Merck Co. (Darmstadt, Germany). All other chemicals were reagent grade and all solvents were HPLC grade.

2.2. Drug administration

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

The rats were randomly divided into three groups (n = 6, each): oral administration of losartan at a dose of 9 mg/kg without or with oral administration

of ticlopidine at a dose of 4 or 10 mg/kg. IV dose was 3mg/kg for absolute bioavilability. The rats were fasted for at least 24 h prior to beginning of the experiments. Each animal was anaesthetized with ether and the right femoral artery (for blood sampling) was cannulated with a polyethylene tube (SP45, I.D. 0.58 mm, O.D. 0.96 mm; Natsume Seisakusho Co. Ltd, Tokyo, Japan).

The losartan solution was diluted in distilled water to make a 1 mg/kg. The ticlopidine was suspended in distilled water. Blood samples (0.5 ml) were collected into heparinized tubes via the femoral artery at 0 (to serve as a control), 0.01(IV), 0.25, 0.5, 0.75(oral), 1, 2, 4, 8, 12 and 24 h after the oral administration of losartan. Blood samples were centrifuged (13,000 rpm, 5 min), and the plasma sample was stored at –40℃ until use for the HPLC analysis of losartan and EXP-3174.

2.3. Method and assay

2.3.1 HPLC Assay

The plasma concentrations of losartan were determined by the HPLC assay modified from Zarghi et al. [26]. Briefly, a 50 μl aliquot of L-158.809 (5 μg/ml dissolved in methanol; an internal standard) and a 0.5 ml aliquot of acetonitril were added to a 0.2 ml aliquot of the plasma sample in a 2.0 ml polypropylene microtube. The mixture was then stirred for 5 min and centrifuged (130,000 rpm, 10 min). A 0.5 ml aliquot of the organic layer was transferred to a clean test tube and evaporated under a gentle stream of nitrogen gas at 35 °C. The residue was reconstituted in a 150 μl aliquot of the mobile phase and centrifuged (13,000 rpm, 5 min). A 70 μl aliquot of the water layer was injected into the HPLC system. The apparatus used in

this study was a high-performance liquid chromatograph equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus autosampler and a Waters™ 474 scanning fluorescence 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), and a high-speed microcentrifuge (Hitachi Co., Tokyo, Japan). The UV detector was set to 215 nm. The stationary phase was a phenomenex C_{18} column (5 μm, 250 × 4.6 mm, Phenomenex co. California, USA) and the mobile phase was acetonitrile: 0.01 M phosphate buffer (41: 59 v/v, pH 2.5, adjusted with phosphoric acid). The retention times at a flow rate of 0.8 ml/min are as follows: internal standard at 6.265 min, losartan at 11.4.12 min and EXP-3174 at 17.828 min. The lower limit of quantification for losartan and EXP-3174 in the rat plasma was 10 ng/ml and 5 ng/ml, respectively. The coefficients of the variation of losartan and EXP-3174 were <13.9% and <15.9%, respectively.

2.3.2 CYP inhibition assay

The inhibition assays on the human CYP3A4 and 2C9 enzyme activity were performed in a multiwell plate using the CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously [28]. Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrates (7-BFC and 7-MFC for CYP3A4 and 2C9, respectively) were incubated with or without test compounds in a reaction mix containing 1 pmol of P450 enzyme and the NADPH generating system (1.3 mM NADP, 3.54mM 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. Metabolite concentrations were measured with a spectrofluorometer (Molecular Device, Sunnyvale, CA) set at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive controls (1 μM ketoconazole and 2 μM sulfaphenazole for CYP3A4 and 2C9, respectively) were run on the same plate and produced 99% inhibition. All experiments were performed in duplicate, and results are expressed as the percent of inhibition.

2.3.3 Rhodamine-123 retention assay

MCF-7/ADR cells were seeded on 24-well plates. At 80% confluence, the cells were incubated in FBS-free DMEM 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 presense of ticlopidine (1, 3, 10 and 30μM) 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. The 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 presented as the ratio to control values.

2.4. Pharmacokinetic analysis

The following pharmacokinetic data were analyzed using the non-compartmental method (WinNonlin software version 4.1; Pharsight Corporation, Mountain View,

CA, USA). The half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and the time to reach peak concentration (t_{max}) of losartan or EXP-3174 were directly read from the experimental data. The area under the plasma concentration time-curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. The relative bioavailability (RB) was estimated by $AUC_{coadmin}/AUC_{control} \times 100$. The absolute bioavailability (AB) was estimated by $AUC_{oral}/AUC_{iv} \times Dose_{iv}/Dose_{oral} \times 100$. The metabolite-parent ratio (MR) was estimated by $AUC_{EXP\text{-}3174}/AUC_{losartan}$

2.5. Statistical analysis

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

3. Results and Discussion

The calibration curves of losartan (Figure 2) and EXP-3174 (Figure 3) were linear within the concentration ranges from 10–200 ng/ml, respectively. The detection limits for losartan and EXP-3174 were 10 ng/ml and 5 ng/ml. The coefficients of the variation of losartan and EXP-3174 were less than 13.9% and 15.9%, respectively (Figure 2 and Figure 3). Figure 5 shows ticlopidine couldn't inhibit p-glycoprotein activitiy.

Figure 6 shows the mean plasma concentration–time profiles of losartan after oral administration (9 mg/kg) with or without of ticlopidine (4 and 10 mg/kg), and intravenous losartan (3mg/kg) without ticlopidine. Table 3 lists the relevant pharmacokinetic parameters of losartan after oral administration. Ticlopidine significantly (4 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased the area under the plasma concentration–time curve (AUC) of losartan and Ticlopidine significantly ($P < 0.05$) increased the peak plasma concentration (C_{max}) of losartan. Total plasma clearance (CL/F) of losartan was decreased significantly (4 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$). Consequently, the relative bioavailability (RB) of losartan increased by 1.30– to 1.72 fold in the presence of ticlopidine. However, there was no significant change T_{max}, volume of distribution (Vdss) and terminal half-life ($t_{1/2}$) of losartan in the presence of ticlopidine.

CYP3A4 and CYP2C9, key enzymes for the metabolism of losartan is mainly located in liver, and in small intestine [7]. The pharmacokinetic studies indicated that losartan is metabolized by cytochrome P450 (CYP) isoenzymes, mainly by

CYP3A4 and CYP2C9 to several active and inactive metabolites [7]. The enhanced bioavailability of losartan by ticlopidine might be due to the inhibition of CYP3A4 and CYP2C9, and decrease of total plasma clearance(CL/F). This result appeared to be consistent with previous studies reported by Piao *et al*. and Choi *et al*. [31, 32]; a single oral administration of morin and naringin significantly increased the AUC and C_{max} of nicardipine and diltiazem in rats, respectively. These results were due to inhibition of CYP3A4 and P-gp in the intestine and/or liver.

Figure 7 depicts the mean plasma concentration–time profiles of EXP-3174 after oral administration of losartan (9 mg/kg) with or without ticlopidine (4 and 10 mg/kg). As listed in Table 4, presence of ticlopidine (10 mg/kg) significantly increased the AUC (41.3%) of EXP-3174 compared with the control group. Metabolite-parent AUC ratio(MR) in the presence of ticlopidine (10 mg/kg) significantly ($p < 0.05$) decreased by 18.2 % compared to the control group, implying that coadministration of ticlopidine could be effective to inhibit the cytochrome P450 (CYP)3A4-and 2C9-mediated metabolism. Presence of ticlopidine did not change the T_{max} and the volume of distribution (Vdss) of EXP-3174. CYPs in enterocytes contribute significantly to the “first-pass” metabolism and oral bioavailability of many drugs and chemicals. The “first pass” metabolism of compounds in the intestine limits absorption of toxic xenobiotics and may ameliorate adverse effects. Moreover, induction or inhibition of intestinal CYPs may be responsible for significant drug/drug interactions when one agent decreases or increases the F and K_a or biotransformation of a concurrently administered drug [32]. The increased bioavailability of losartan by ticlopidine suggests that CYP3A4 and 2C9 could be inhibited by ticlopidine, which resulted in reducing first-pass

metabolism of losartan in the intestine and/or liver. Furthermore, another reason for increased bioavailability of losartan is the decrease of total body clearance (CL/F) of losartan by ticlopidine. We investigated a cell-based P-gp activity using rhodamne-123, because losartan is the substrate of P-gp, and the result showed that ticlopidine did not affect P-gp activity.

Therefore, concomitant use of ticlopidine with losartan will require close monitoring to potential drug interactions for the safe therapy of cardiovascular diseases. Clinical importance of these finding should be further investigated in clinical trials.

4. Conclusion

Presence of ticlopidine significantly enhanced the bioavailability in rats. The increased bioavailability was due to inhibition of the CYP3A4- and CYP2C9-mediated metabolism of losartan in the small intestine or in the liver, and furthermore, due to decreased total body clearance of losartan. If the results are further confirmed in the clinical trial, dose adjustment of losartan should be taken into consideration when losartan is administered concomitantly with ticlopidine to the patients.

References

[1] D. Javier. Review of the Molecular Pharmacology of Losartan and Its Possible Relevance to Stroke Prevention in Patients with Hypertension. Clinical Therapeutics. 2006; 28; 832-848.

[2] M. McIntyre, S. E. Caffe, R. A. Michalak, J. L. Reid. Losartan, an orally active angiotensin (AT1) receptor antagonist: a review of its efficacy and safety in essential hypertension. Pharmacol Ther. 1997; 74; 181-194.

[3] T. Inagami, N. Iwai, K. Sasaki, Y. Yamamo, S. Bardhan, S. Chaki, D. F. Guo, H. Furuta. Cloning, expression and regulation of angiotensin II receptors. J Hypertens. 1992; 8; 713-716.

[4] M. W. Lo, M. R. Goldberg, J. B. McCrea, H. Lu, C. I. Furtek, T. D. Bjornsson. Pharmacokinetics of losartan, an angiotensin II receptor antagonist, and its active metabolite EXP-3174 in humans, Clin Pharmacol Ther. 1995; 58; 641-649.

[5] A. Soldner, S. L. Hildegard, E. Mutschler. HPLC assays to simultaneously determine the angiotensin-AT1 antagonist losartan as well as its main and active metabolite EXP-3174 in biological material of humans and rats, Journal of Pharmaceutical and Biomedical Analysis. 1998; 16; 863-873.

[6] A. Soldner, U. Christians, M. Susanto, V. J. Wacher, J. A. Silverman, L. Z. Benet. Grapefruit juice activates P-glycoprotein-mediated drug transport. Pharm. Res. 1999; 16; 478-485.

[7] R. A. Stearns, P. K. Chakravarty, R. Chen, S. H. Chiu. Biotransformation of

losartan to its active carboxylic acid metabolite in human liver microsomes. Role of cytochrome P4502C and 3A subfamily members. Drug Metab. Dispos. 1995; 23; 207-215.

[8] R. A. Stearns, R. R. Miller, G. A. Doss, P. K. Chakravarty, A. Rosegay, G. J. Gatto, S. H. Chiu. The metabolism of DuP 753, a nonpeptide angiotensin II receptor antagonist, by rat, monkey, and human liver slices. Drug. Metab. Dispos. 1992; 20; 281-287.

[9] C. H. Yun, H. S. Lee, H. Lee, J. K. Rho, H. G. Jeong, F. P. Guengerich. Oxidation of the angiotensin II receptor antagonist losartan (DuP 753) in human liver microsomes. Role of cytochrome P4503A(4) in formation of the active metabolite EXP3174. Drug. Metab. Dispos. 1995; 23; 285-289.

[10] A. M. Meadowcroft, K. M. Williamson, J. H. Patterson, A. L. Hinderliter, J. A. Pieper. The effects of fluvastatin, a CYP2C9 inhibitor, on losartan pharmacokinetics in healthy volunteers. J. Clin. Pharmacol. 1999; 39; 418-424.

[11] K. M. Kaukonen, K. T. Olkkola, P. J. Neuvonen. Fluconazole but not itraconazole decreases the metabolism of losartan to E-3174. Eur. J. Clin. Pharmacol. 1998; 53; 445-449.

[12] J. B. McCrea, A. Cribb, T. Rushmore, B. Osborne, L. Gillen, M. W. Lo, S. Waldman, T. Bjornsson, S. Spielberg, M. R. Goldberg. Phenotypic and genotypic investigations of a healthy volunteer deficient in the conversion of losartan to its active metabolite E-3174. Clin. Pharmacol. Ther. 1999; 65; 348-352.

[13] V. J. Wacher, L. Salphati, L. Z. Benet. Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv. Drug. Deliv. Rev. 2001; 46; 89-

102.

[14] M. M. Gottesman, I. Pastan. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu. Rev. Biochem. 1993; 62; 385-427.

[15] L. S. L. Gan, M. A. Moseley, B. Khosla, P. F. Augustijns, T. P. Bradshaw, R. W. Hendren, D. R. Thakker. CYP3A-Like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells: interaction between the two biochemical barriers to intestinal transport. Drug. Metab. Dispos. 1996; 24; 344-349.

[16] V. H. Wacher, J. A. Silverman, Y. Zhang, L. Z. Benet. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. J Pharm Sci. 1998; 87; 1322-1330.

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

[18] Saltiel E, Ward A. Ticlopidine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutics efficacy in platelet-dependent disease states. Drugs 1987; 34; 222-262.

[19] Gent M, Blakely JA, Easton JD, Ellis DJ, Hachinski VC, Harbison JW, et al. The Canadian American ticlopidine study (CATS) in thromboembolic stroke. Lancet 1989; 1; 1215-1220.

[20] Hass Wk, Easton JD, Adams HP, Pryse-Phillips W, Molony BA, Anderson S, Kamm B. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. N Engl J Med 1989; 321; 501-507.

[21] Janzon L, Bergqvist D, Boberg J, Boberg M, Eriksson I, Lindgarde F, Persson G. Prevention of myocardial infarction and stroke in patients with intermittent claudication; effects of ticlopidine. Results from STIMS, the Swedish Ticlopidine Multicentre Study. J Intern Med 1990; 227; 301-308.

[22] Haynes RB, Sandler RS, Larson EB, Pater JL, Yatsu FM. A critical appraisal of ticlopidine, a new antiplatelet agent. Effectiveness and clinical indications for prophylaxis of atherosclerotic events. Arch Intern Med 1998; 152; 1376-1380.

[23] Ito MK, Smith AR, Lee ML. Ticlopidine: a new platelet aggregation inhibitors. Clin Pharm 1992; 11; 603-617

[24] Verhaeghe R. Prophylactic antiplatelet therapy in peripheral arterial disease. Drugs 1991; 42; 51-57.

[25] Solomon DH, Hart RG. Antithrombotic therapies for stroke prevention. Curr Opin Neurol 1994; 7; 48-53.

[26] Buur T, Larsson R, Berglund U, Donat F, DVM, Tronquet C. Pharmocokinetics and effect of ticlopidine on platelet aggregation in subjects with normal and impaired renal function. J Clin Pharmacol 1997; 37; 108-115.

[27] Ko JW, Desta Z, Soukhova NV, Tracy T, Flockhart DA. In vitro inhibition of the cytochrome P450 (CYP450) system by the antiplatelet drug ticlopidine: potent effect on CYP2C19 and CYP3A4. Br J Clin Pharmacol. 2000; 49; 343-351.

[28] A. Zarghi, S. M. Foroutan, A. Shafaati, A. Khoddam. A rapid HPLC method for the determination of losartan in human plasma using a monolithic column. Arzneimittelforschung. 2005; 55; 569-572.

[29] Crespi CL, Miller VP, Penman BW: Microtiter plate assays for inhibition of

human, drug-metabolizing cytochromes P450. Anal Biochem, 1997, 248; 188–190.

[30] S. P. Hong, K. S. Chang, D. H. Choi, J. S. Choi. Effect of atorvastatin on the pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats. Arch Pharm Res. 2007; 30; 90-95.

[31] Y. J. Piao, J. S. Choi. Effects of morin on the pharmacokinetics of nicardipine after oral and intravenous administration of nicardipine in rats. J Pharm Pharmacol. 2008; 60; 625-629.

[32] J. S. Choi, H. K. Han. Enhanced oral exposure of diltiazem by the concomitant use of naringin in rats. Int J Pharm. 2005; 305; 122-128.

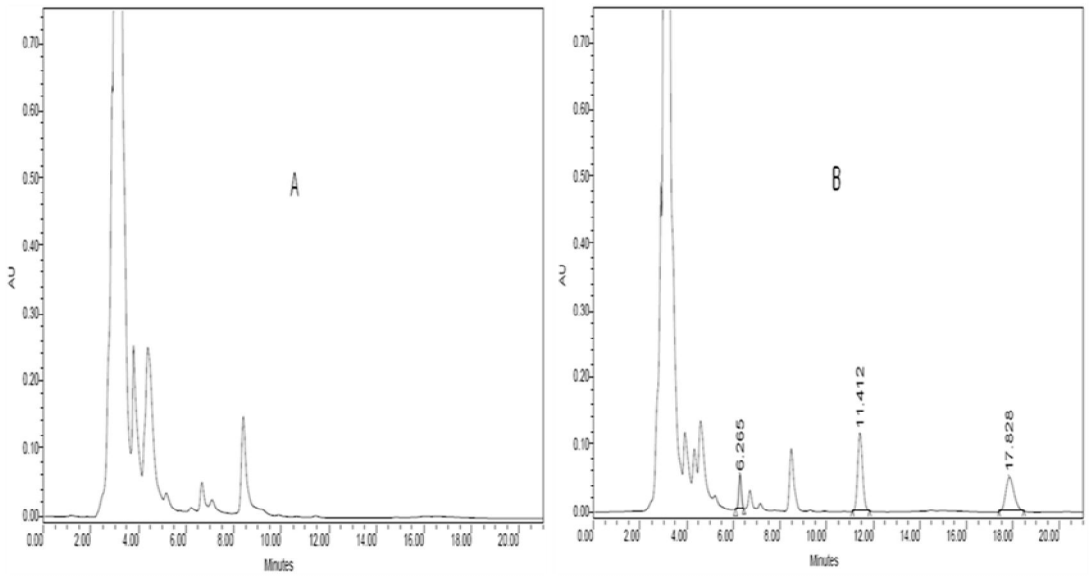

Figure 1. HPLC chromatograms of the rat's blank plasma (A), and the plasma spiked with losartan (11.412 min), EXP-3174 (17.828 min), and L-158.809 (internal standard; 6.265 min) (B).

Figure 1. HPLC chromatograms of the rat's blank plasma (A), and the plasma spiked with losartan (11.412 min), EXP-3174 (17.828 min), and L-158.809 (internal standard; 6.265 min) (B).

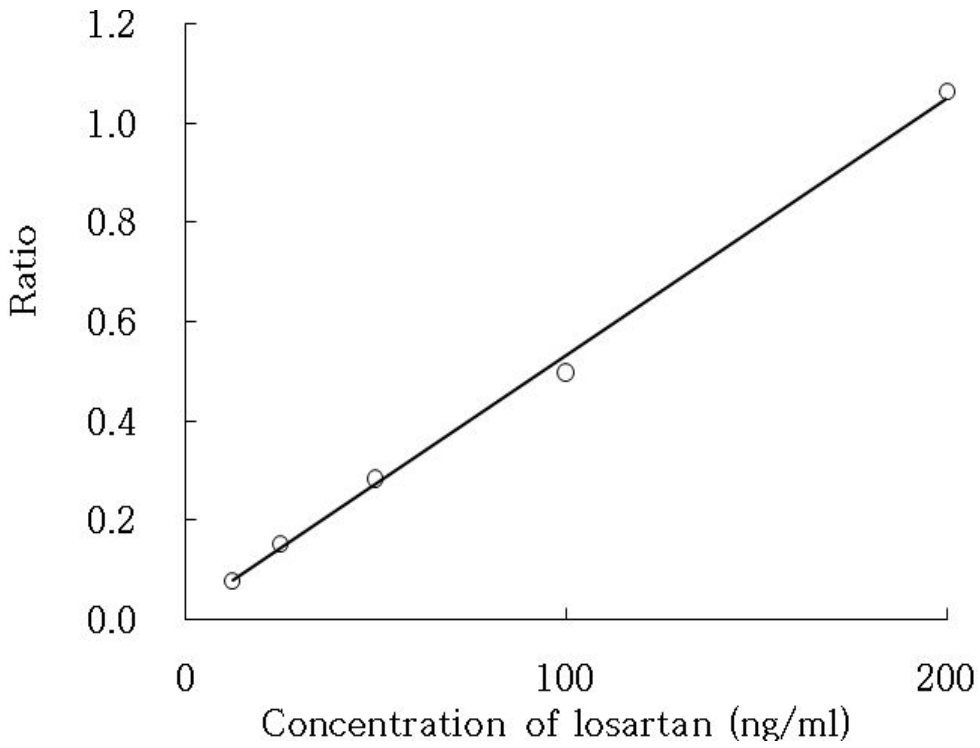

Figure 2. A calibration curve of losartan when spiked into the rat's blank plasma.

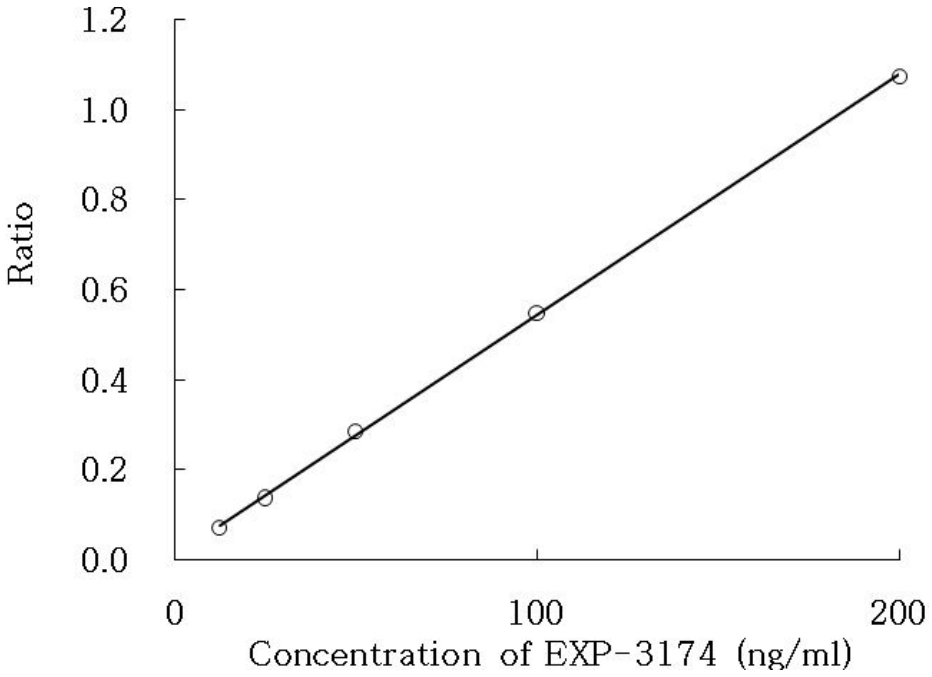

Figure 3. A calibration curve of EXP-3174 when spiked into the rat's blank plasma.

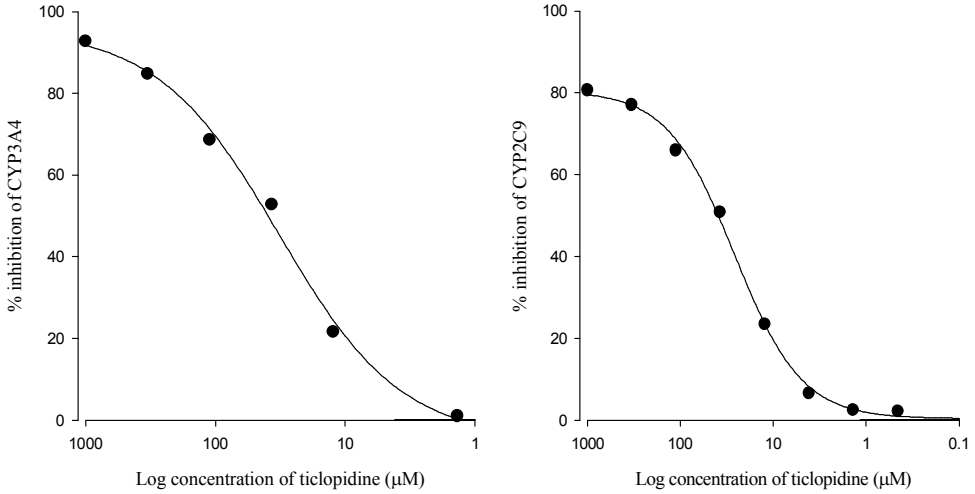

Fig. 4**.** Inhibitory effect of ticlopidine on CYP3A4 (A) and 2C9 (B) activity. All experiments were performed in duplicate, and results are expressed as the percent of inhibition. (IC_{50}: CYP3A4; 32.3μM, CYP2C9; 26.0μM)

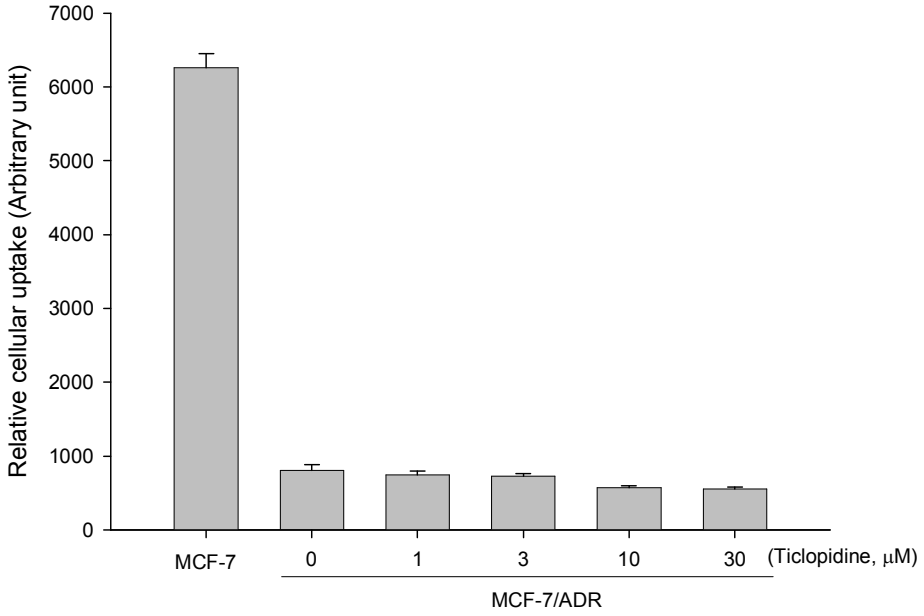

Fig. 5. Rhodamine-123 retention. MCF-7/ADR cells were incubated with ticlopidine for 24h. After incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min, the R-123 fluorescence values in cell lysates were measured using excitation and emission wavelengths of 480 and 540 nm, respectively. The values were divided by the total protein content of each sample. Data represents mean ± SD of 6 separate samples.

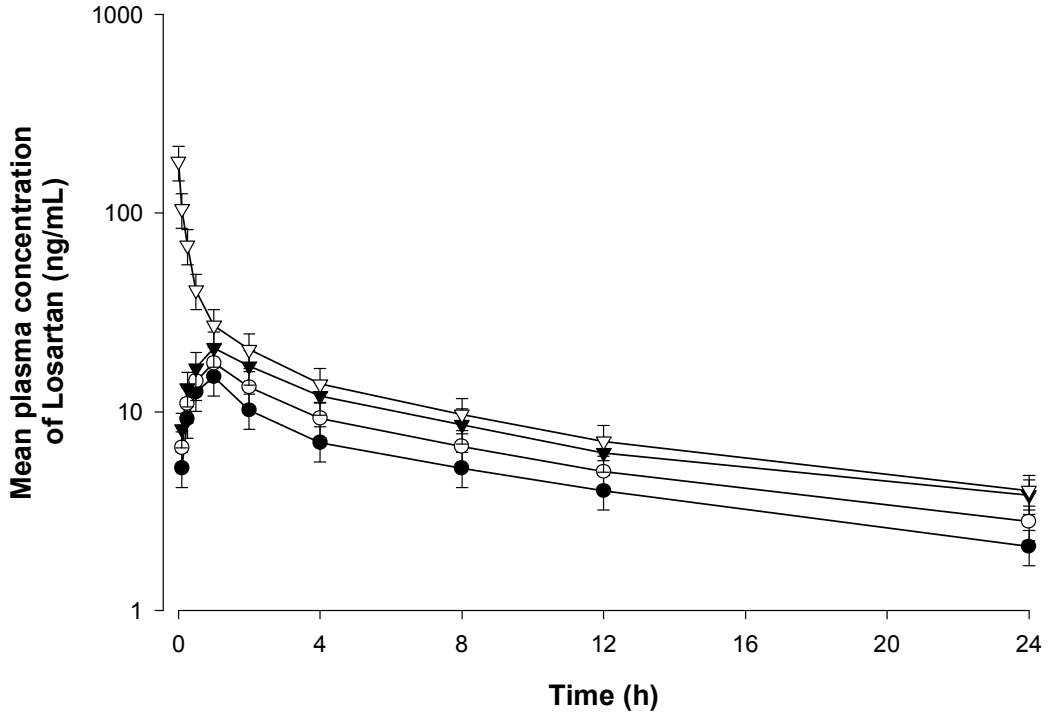

Figure 6. Mean arterial plasma concentration–time profiles of losartan after oral administration of losartan (9 mg/kg) without (●) or with 4 mg/kg (○) or 10 mg/kg (▼) and intravenous of losartan (3 mg/kg) without ticlopidine (▽) to rats (mean±SD, n = 6)

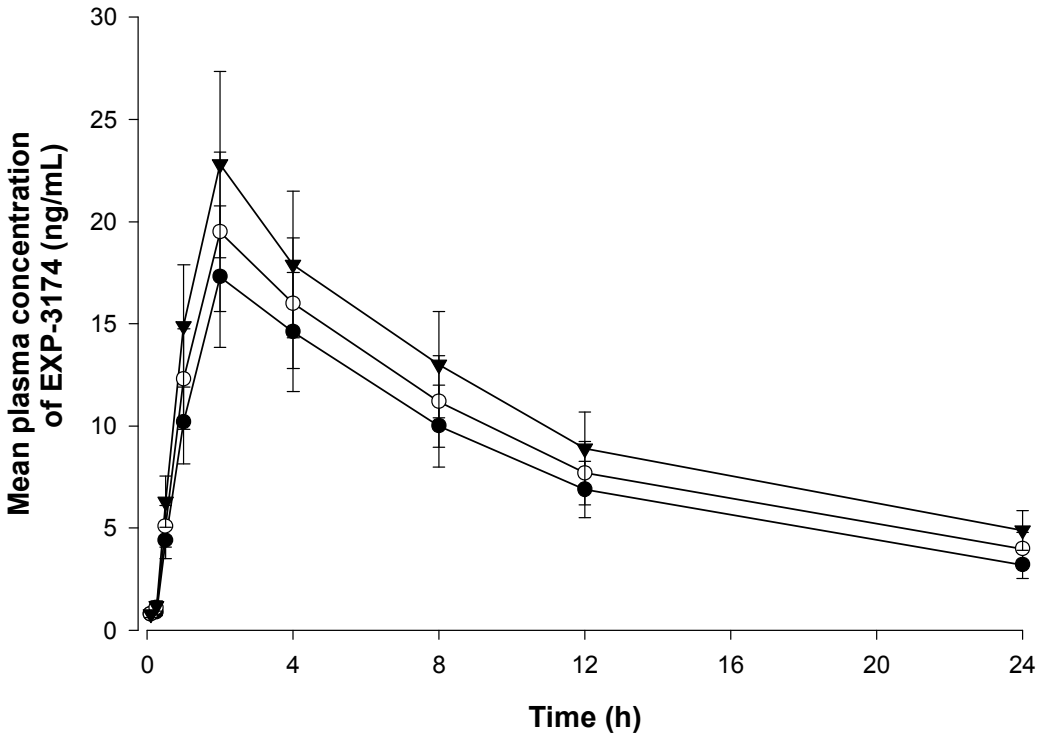

Figure 7. Mean arterial plasma concentration–time profiles of EXP-3174 after oral administration of losartan (9 mg/kg) without (●) or with 4 mg/kg (○) or 10 mg/kg (▼) of ticlopidine to rats (mean±SD, n = 6)

Table 1. Mean (± S.D.) plasma concentrations of losartan after oral administration of losartan (9 mg/kg) without or with ticlopidine to rats (n = 6, each).

Time (h)	Control (Losartan)	Losartan + Ticlopidine		Losartan
		4 mg/kg	10 mg/kg	IV
0	0	0	0	181.4±32.65
0.1	5.2±1.04	6.6±1.25	8.2±1.48	104.8±18.86
0.25	9.2±1.84	11±2.09	13.2±2.38	68.9±12.40
0.5	12.6±2.52	14.3±2.72	16.6±2.99	41±7.38
1	15±3.00	17.6±3.34	21±3.78	27.2±4.90
2	10.2±2.04	13.3±2.53	17±3.06	20.6±3.71
4	7±1.40	9.3±1.77	12±2.16	13.8±2.48
8	5.2±1.04	6.7±1.27	8.6±1.55	9.7±1.75
12	4±0.80	5±0.95	6.2±1.12	7.1±1.28
24	2.1±0.42	2.8±0.53	3.8±0.68	4.0±0.72

Table 2. Mean (± S.D.) plasma concentrations of EXP-3174 after oral administration of losartan (9 mg/kg) with or without of ticlopidine to rats (n = 6, each).

Time (h)	Control (EXP-3174)	Losartan + Ticlopidine	
		4 mg/kg	10 mg/kg
0	0	0	0
0.1	0.8±0.16	0.8±0.15	0.8±0.14
0.25	0.9±0.18	1.1±0.21	1.2±0.22
0.5	4.4±0.88	5.1±0.97	6.3±1.13
1	10.2±2.04	12.3±2.34	14.9±2.68
2	17.3±3.46	19.5±3.71	22.8±4.10
4	14.6±2.92	16±3.04	17.9±3.22
8	10±2.01	11.2±2.13	13±2.34
12	6.9±1.38	7.7±1.46	8.9±1.60
24	3.2±0.64	4±0.76	4.9±0.88

Table 3. Mean (± S.D.) pharmacokinetic parameters of losartan after oral administration(9 mg/kg) and intravenous administration(3 mg/kg) of losartan with or without of ticlopidine to rats (n = 6, each).

Parameter	Control	Losartan + Ticlopidine		Losartan
		4 mg/kg	10 mg/kg	IV
AUC (ng·h/ml)	156±28.08	204±36.72*	270±48.57**	330±59.41
C_{max} (ng/ml)	15±3.25	17.6±3.64	21±4.16*	-
T_{max} (h)	1	1	1	-
$t_{1/2}$ (h)	12.4±2.48	13.0±2.54	14.2±2.56*	11.4±1.60
Kel(h^{-1})	0.058±0.0116	0.059±0.0118	0.060±0.0119	0.061±0.0201
CL/F(mL/min/kg)	962.6±186.64	736.3±155.17*	555.9±124.50*	149.1±39.35
Vdss(L/min/kg)	13.5±4.18	11.0±3.68	9.5±3.50	-
AB (%)	15.8±3.05	20.6±3.68*	27.3±4.55**	100
RB (%)	100	130	172	-

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

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

C_{max}: peak plasma concentration.

T_{max}: time to reach the peak plasma concentration.

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

Kel: elimination rate constant.

CL/F: total plasma clearance.

Vdss: volume of distribution at the steady state.

AB: absolute bioavailability.

RB: relative bioavailability.

Table 4. Mean (± S.D.) Pharmacokinetic parameters of EXP-3174 after oral administration of losartan (9mg/kg) with or without of ticlopidine to rats (*n* = 6, each).

Parameter	Control	Losartan + Ticlopidine	
		4 mg/kg	10 mg/kg
AUC (ng·h/ml)	232±39.44	272±46.24	328±55.76*
C_{max} (ng/ml)	17.3±2.81	19.5±3.35	22.8±3.74
T_{max} (h)	2	2	2
$t_{1/2}$ (h)	9.02±1.77	9.69±1.83	10.9±2.01
Kel(h^{-1})	0.081±0.0155	0.073±0.0142	0.058±0.0121
CL/F(mL/min/kg)	647.8±121.46	552.5±99.45*	457.7±91.29*
Vdss(L/min/kg)	7.9±1.61	7.2±1.41	6.5±1.31
M.R(%)	148±26	133±24	121±21*

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

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

C_{max}: peak plasma concentration.

T_{max}: time to reach the peak plasma concentration.

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

Kel: elimination rate constant.

CL/F: total plasma clearance.

Vdss: volume of distribution at the steady state.

M.R: metabolite-parent ratio.

Acknowledgements

지난 2 년간의 대학원 생활에서 여러가지로 부족한 저를 이끌어주시고, 힘들 때마다 격려해주시고, 실수할 때는 충고를 아끼지 않으신 지도 교수님, 최준식 교수님께 무한한 감사를 드립니다.

또한 항상 뒤에서 지켜봐주시는 한효경 교수님, 강건욱 교수님께 감사의 말씀을 전하고 싶습니다.

힘든 대학원 생활 동안 저의 힘이 되어준 선배 이성 그리고 동기 서상완, 고운정에게도 깊은 감사의 말씀 드립니다.

마지막으로 어려운 상황 속에서 항상 제 버팀목이 되어주고 힘을 실어준 사랑하는 부모님, 그리고 동생 순기에게, 앞으로는 제가 우리가족의 버팀목이 될 것을, 그리고 사랑하고 감사하다고 전해드리고 싶습니다.

저작물 이용 허락서

학 과	약학과	학 번	20087229	과 정	석사
성 명	한 글 : 김형기 한 문 : 金亨基 영 문 : Hyung-ki Kim				
주 소	서울특별시 양천구 목 5 동 929 한신@ 110-1304				
연락처	E-mail : khk8511@hanmail.net				
논문제목	한글: 흰쥐에서 로살탄과 티크로피딘과의 상호작용 영문: Pharmacokinetic interaction between Losartan and Ticlopidine in rats.				

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

- 다 음 -

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

동의여부 : 동의 (√) 반대 ( )

20010 년 2 월

저작자: 김형기 (서명 또는 인)

조선대학교 총장 귀하