2011 년 2 월
석사학위논문

Pharmacokinetic Interaction between Nifedipine and Warfarin in Rats

조 선 대 학 교 대 학 원

약 학 과

박 선 희

Pharmacokinetic Interaction between Nifedipine and Warfarin in Rats

흰쥐에서 니페디핀과 왈파린과의
약물동태학적 상호작용

2011 년 2 월

조 선 대 학 교 대 학 원

약 학 과

박 선 희

Pharmacokinetic Interaction between Nifedipine and Warfarin in Rats

지도교수 최 준 식

이 논문을 약학석사학위신청 논문으로 제출함.

2010년 10월

조 선 대 학 교 대 학 원

약 학 과

박 선 희

박선희의 석사학위논문을 인준함

위원장 조선대학교 교수 최후균 인
위 원 조선대학교 교수 강건욱 인
위 원 조선대학교 교수 최준식 인

2010년 11월

조 선 대 학 교 대 학 원

CONTENTS

Abstract 1
국문초록.....3
1. Introduction.....5
2. Materials and Methods.....7
2.1. Chemicals and apparatus.....7
2.2. Animal experiments.....7
2.3. Oral and intravenous administration of warfarin.....8
2.4. Method and assay.....8
2.4.1. HPLC assay.....8
2.4.2. CYP inhibition assay.....9
2.4.3. Rhodamine-123 retention assay.....10
2.5. Pharmacokinetic analysis.....10
2.6. Statistical analysis.....11
3. Results.....12
3.1. Inhibitory effect of nifedipine on CYP3A4 activity.....12
3.2. Rhodamine-123 retention assay.....12
3.3. Effect of nifedipine on the pharmacokinetics of warfarin after oral administration.....12
3.4. Effect of nifedipine on the pharmacokinetics of warfarin after intravenous administration.....13
4. Discussion.....14
5. Conclusion.....16

References……17

LIST OF FIGURES

Figure 1. HPLC chromatograms of the rat's blank plasma (A), and the plasma spiked with warfarin and 7-ethoxycoumarin (B)……21

Figure 2. Inhibitory effect of nifedipine on CYP3A4 activity. All experiments were performed in duplicate, and results were expressed as the percent of inhibition (IC_{50}: 9.1 μM)……22

Figure 3. Rhodamine-123 retention. MCF-7/ADR cells were preincubated with nifedipine for 30 min, and after incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min. The rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. The values were divided by total protein contents of each sample. Verapamil (100 μM) was used as a positive control. Data represents mean ± SD of 6 separate samples……23

Figure 4. Mean arterial plasma concentration–time profiles of warfarin after oral administration of warfarin (0.2 mg/kg) without (●) or with 0.5 mg/kg (○) and 2 mg/kg (▼) of nifedipine in rats. Bars represent the standard deviation (n = 6) ……24

Figure 5. Mean arterial plasma concentration–time profiles of warfarin after intravenous administration of warfarin (0.05mg/kg) without (●) or with 0.5 mg/kg (○) and 2 mg/kg (▼) of nifedipine in rats. Bars represent the standard deviation (n = 6)……25

LIST OF TABLES

Table 1. Mean plasma concentration of warfarin after oral administration of warfarin (0.2 mg/kg) with or without nifedipine (0.5 and 2 mg/kg) in rats (Mean ± SD, *n* = 6)……26

Table 2. Mean plasma concentration of warfarin after intravenous administration of warfarin (0.05 mg/kg) with or without nifedipine (0.5 and 2 mg/kg) in rats (Mean ± SD, *n* = 6)……27

Table 3. Mean pharmacokinetic parameters of warfarin after oral administration of warfarin (0.2 mg/kg) with or without nifedipine (0.5 and 2 mg/kg) in rats (Mean ± SD, *n* = 6)……28

Table 4. Mean pharmacokinetic parameters of warfarin after intravenous administration of warfarin (0.05 mg/kg) with or without nifedipine (0.5 and 2 mg/kg) in rats (Mean ± SD, *n* = 6)……29

Pharmacokinetic Interaction between Nifedipine and Warfarin in Rats

By : Sun-hee Park
Advisor : Prof. Jun-Shik Choi, Ph.D.
Department of Pharmacy,
Graduate School of Chosun University

Abstract

The aim of this study was to investigate the effect of nifedipine on the pharmacokinetics of warfarin after oral and intravenous administration of warfarin in rats. Warfarin was administered orally (0.2 mg/kg) or intravenously (0.05 mg/kg) without or with oral administration of nifedipine (0.5 or 2 mg/kg) in rats. The effect of nifedipine on the P-glycoprotein (P-gp) as well as cytochrome P450 (CYP) 3A4 activity was also evaluated. Nifedipine inhibited CYP3A4 enzyme activity with 50 % inhibition concentration (IC_{50}) of 9.1 μM. Compared to those animals in the oral control group (warfarin without nifedipine), the area under the plasma concentration–time curve (AUC) of warfarin was significantly greater (2 mg/kg, $p < 0.05$) by 13.1–36.0 %, and the peak plasma concentration (C_{max}) was significantly higher (2 mg/kg, $p < 0.05$) by 24.7 % after oral administration of warfarin with

nifedipine, respectively. Consequently, the relative bioavailability (R.B.) of warfarin increased by 1.13- to 1.36-fold and the absolute bioavailability (A.B.) of warfarin with nifedipine was significantly greater by 59.0–70.9 % compared to that in the control group (52.1 %). In contrast, nifedipine had no effect on any pharmacokinetic parameters of warfarin given intravenously. Therefore, the enhanced oral bioavailability of warfarin may be due to inhibition of CYP 3A4-mediated metabolism in the intestine and/or liver rather than elimination, resulting in reducing first-pass metabolism by nifedipine.

Key words: Warfarin, Nifedipine, Pharmacokinetics, Bioavailability, CYP3A4, P-glycoprotein, Rats

국 문 초 록

흰쥐에서 니페디핀과 왈파린과의 약물동태학적 상호작용

박 선 희

지 도 교 수 : 최 준 식

조선대학교 대학원 약학과

본 연구는 임상에서 항고혈압제인 니페디핀이 심혈전증 및 관상동맥 폐색증 치료제인 왈파린약물과의 병용처방이 가능하다. 그러므로 흰쥐에서 니페디핀과 왈파린을 병용투여시 왈파린의 생체이용률 및 약물동태에 미치는 영향을 검토 하였다. 왈파린의 약물동태 파라미터는 니페디핀 (0.5 및 2 mg/kg) 과 왈파린 경구 (0.2 mg/kg) 및 정맥 (0.05 mg/kg) 투여 하여 본연구를 실시하였다. 니페디핀이 p-glycoprotein (p-gp), CYP3A4의 활성에 미치는 영향을 평가하였다. 니페디핀의 CYP3A4의 50% 효소활성억제는 9.1 uM 이었다. 그리고 니페디핀은 MCF-7/ADR 세포의 로다마인123 세포 축적을 유의성 있게 증가 시켰다. 니페디핀는 왈파린의 혈장곡선하면적(AUC)은 13.1-36.0% 증가 시켰다. 따라서 왈파린의 절대적생체이용률(A.B.)는 니페디핀과 병용시 59.0-70.9% 증가시켰으며, 이는 왈파린의 상대적 생체이용률(R.B.)에 대조군은 1.13-1.36배 증가되었다. 반감기는 유의성 있게 연장되었다. 그러나 니페디핀과 병용시에 최고혈중농도(C_{max})에 도달시간과

전신크리어런스에는 변화가 없었다.

정맥투여시 니페디핀 (2 mg/kg) 은 왈파린의 AUC (21 %)를 증가시켰으나 유의성은 없었다. 그리고 니페디핀과 병용시 전신크리어런스와 반감기에는 변화가 없었다. 니페디핀이 왈파린의 생체이용률을 증가시킨 것은 니페디핀이 P-gp의 억제와 소장과 간장에서 CYP3A4 활성을 억제 시켜 왈파린의 초회통과효과를 감소시킨 결과 때문으로 사료된다.

결과적으로 니페디핀은 왈파린의 생물학적 이용률를 증가시킨다는 점에서 이들약물의 병용투여시 안전역이 좁은 왈파린의 약물상호작용 가능성을 면밀히 검토 하는 것이 필요하다고 사료된다.

1. Introduction

Nifedipine, a dihydropyridine calcium channel antagonist, causes coronary and peripheral vasodilatation by blocking the influx of extracellular calcium across cell membranes. Nifedipine has been used for the treatment of hypertension, myocardial ischemia, and related cardiovascular disorders [1, 2]

Nifedipine is rapidly and almost completely absorbed from the gastrointerstinal tract, but undergoes extensive hepatic first-pass metabolism. Nifedipine is about 92 to 98% bound to plasma proteins. It is extensively metabolized in the liver and 70 to 80% of a dose is excreted in the urine almost entirely as inactive metabolites. The half-life is about 2 hours following intravenous administration or administration of capsules [3-5].

It is a substrate of cytochrome P450 (CYP) 3A subfamily, especially CYP3A4 in humans and forms to pharmacologically inactive metabolite [6, 7]. In addition, nifedipine is a P-glycoprotein (P-gp) substrate [8, 9].

Warfarin is the most extensively used oral anticoagulant for the prevention and treatment of thromboembolic complications in cardiovascular diseases such as atrial fibrillation, venous thrombosis and pulmonary embolism [10]. Warfarin's anticoagulant effect is due to its interference with the cyclic interconversion of vitamin K and its 2, 3 epoxide, and to its limitation of the synthesis of the vitamin K-dependent clotting factors, II, VII, IX and X [11, 12]. Warfarin is readily absorbed from the gastrointestinal tract, extensively bound to plasma proteins. Warfarin is used as a racemic mixture of roughly equal amounts of R and S enantiomers yet S-warfarin has been reported to be more potent [13]. Warfarin is metabolized by CYPs and is converted to inactive metabolites through selective hydroxylation [14]. R-warfarin is metabolized primarily by CYP3A4 to 10-hydroxywarfarin, by CYP1A2 to 6- and 8-hydroxywarfarin, and by carbonyl reductases to

diastereoisomeric alcohols. On the other hand, S-warfarin is metabolized primarily by CYP2C9 to 7-hydroxywarfarin. Potential warfarin-drug interactions could occur with any of a very wide range of drugs that are metabolized by these CYPs, and a number of such interactions have been reported [16-23].

However, there have been no reports regarding the effects of nifedipine on the bioavailability or pharmacokinetics of warfarin in rats. Moreover, nifedipine and warfarin could be prescribed for the prevention or treatment of thromboembolism and ischemic stroke in some patients with atrial fibrillation, valvular heart disease and a myocardial infarction as a combination therapy. Warfarin has a narrow therapeutic range and its efficacy can be influenced by drug-drug interactions. Therefore, the present study aimed to investigate the effects of nifedipine on the pharmacokinetics of warfarin after oral and intravenous administration in rats.

2. Materials and methods

2.1. Chemicals and apparatus

Warfarin, nifedipine and 7-ethoxycoumarin (internal standard for high-performance liquid chromatograph analysis of warfarin) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC grade acetonitrile was a purchased from Merck Co. (Darmstadt, Germany). Other chemicals for this study were reagent grade.

The HPLC system used in this study was comprised of a Waters 1515 isocratic HPLC pump, a Waters 717 plus autosampler and a Waters™ 474 scanning fluorescence detector (Waters Co., Milford, MA, USA), a 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 micro centrifuge (Hitachi Co., Tokyo, Japan).

2.2. Animal experiments

Male Sprague–Dawley rats 7–8 weeks of age (weighing 270–300 g) were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Republic of Korea) and given free access to a commercial rat chow diet (No. 322-7-1; Superfeed Co., Gangwon, Republic of Korea) and tap water. The animals were housed, two per cage, maintained at 22 ± 2°C and 50–60 % relative humidity under a 12:12 h light-dark cycle. The rats were acclimated under these conditions for at least 1 week. All animal studies were performed in accordance with

the “Guiding Principles in the Use of Animals in Toxicology” adopted by the Society of Toxicology (USA) and the Animal Care Committee of Chosun University (Gwangju, Republic of Korea) approved the protocol of this animal study. The rats were fasted for at least 24h prior to beginning the experiments and had free access to tap water. Each animal was anaesthetized with light 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 drug administration, respectively.

2.3. Oral and intravenous administration of warfarin

The rats were randomly divided into four groups (n = 6, each); an oral group (0.2 mg/kg of warfarin dissolved in water; homogenized at 36^{o}C for 30 min; 1.0 mL/kg) without (control) or with 0.5 or 2 mg/kg of oral nifedipine, and an intravenous group (0.05 mg/kg of warfarin, dissolved in 0.9 % NaCl-injectable solution; homogenized at 36^{o}C for 30 min; 0.3 mL /kg) without (control) or with 0.5 or 2 mg/kg of oral nifedipine. Warfarin was administered orally using a gastric gavage tube, and nifedipine was orally administered 30 min prior to oral or intravenous administration of warfarin. Warfarin for intravenous administration was injected through the femoral vein within 0.5 min. A blood sample (0.3 mL) was collected into heparinized tubes from the femoral artery at 0 (control), 0.25, 0.5, 1, 2, 4, 8, 12, 24 and 36 h after intravenous infusion, and 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48 and 72 h after oral administration. The blood samples were centrifuged (13,000 rpm, 5 min), and the plasma samples (0.15 mL) were stored at –40˚C until HPLC analysis of warfarin. An approximately 1 mL of whole blood collected from untreated rats was infused via the femoral artery at 0.25, 1, 3 and 8 h, respectively, to replace blood loss due to blood sampling.

2.4. Method and assay

2.4.1 HPLC Assay

The plasma concentrations of warfarin were determined by a HPLC assay method reported by Zhu et al [24]. Briefly, 50 μL of 7-ethoxycoumarin (2 μg/mL dissolved in methanol), 50 μL of methanol, 200 mL distilled water, 0.5 mL of 2 M hydrochloric acid, and 0.8 mL of diethyl ether were added to 0.15 mL of plasma sample. The mixture was then stirred for 10 min and centrifuged (13,000 rpm, 10 min). 0.7 mL of the organic layer was transferred to a clean test tube and evaporated under a gentle stream of nitrogen at 35°C. The residue was dissolved in 150 μL of the phosphate buffer. Next, 70 μL of the supernatant was injected into the HPLC system. Chromatographic separations were achieved using a guard column packed with C_{18} column (4 × 3.0 mm, 5 μm, Phenomenex), and a reversed-phase Luna® C_{18} column (4.6 × 150 mm, 5 μm, Phenomenex). The mobile phase was 10 mM phosphate buffer–methanol (50:50, v/v), which was run at a flow rate of 1.0 mL/min. Chromatography was performed at a temperature of 30˚C that was set by an HPLC column temperature controller, while the UV detector was set to 300 nm. The retention times of warfarin and the internal standard were 16.7 and 9.1 min, respectively (Fig. 2). The detection limit of warfarin in rat's plasma was 5 ng/mL. The coefficients of variation for warfarin were below 12.8 %.

2.4.2 CYP inhibition assay

The inhibition assays of human CYP3A4 enzyme activity were performed in a multiwell plate using the CYP inhibition assay kit (BD Bioscience, San Jose, CA). Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP3A4 substrate (7-Benzyloxy-4-(trifluoromethyl)couamrin (BFC)) was incubated with or without test compounds in a reaction mixture containing 1 pmol

of CYP3A4 enzyme and the NADPH-generating system (1.3 mM NADP, 3.54 mM glucose 6-phosphate, 0.4 U/mL glucose 6-phosphate dehydrogenase and 3.3 mM $MgCl_2$) 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 control (1 μM ketoconazole) was run on the same plate and produced 99 % inhibition. All experiments were performed in duplicate, and the results are expressed as the percent of inhibition.

2.4.3 Rhodamine-123 retention assay

The P-gp-overexpressed multidrug resistant human breast carcinoma cell line (MCF-7/ADR cells) was seeded in 24-well plates. At 80 % confluence, the cells were incubated in fetal bovine serum (FBS)-free Dulbecco's modified Eagle's medium (DMEM) for 18 h. The culture medium was changed with 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 nifedipine (1, 3 or 10 μM) or verapamil (100 μM) for 90 min, the medium was completely aspirated. The cells were then washed three times with an 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 percentage ratio to control.

2.5. Pharmacokinetic analysis

The pharmacokinetic parameters were calculated using non-compartmental

analysis (WinNonlin; software version 4.1; Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of warfarin concentration data during the elimination phase, and 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 warfarin 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}. Total body clearance (CL) was calculated by Dose/AUC. The absolute bioavailability (A.B.) of warfarin was calculated by $AUC_{oral}/AUC_{intravenous} \times Dose_{intravenous}/Dose_{oral} \times 100$, and the relative bioavailability (R.B.) of warfarin was estimated by $AUC_{with\ nifedipine}/AUC_{control} \times 100$.

2.6. Statistical analysis

All data were expressed with their standard deviation (mean ± SD). Statistical analysis was conducted using a one-way analysis of variance (ANOVA) followed by a posteriori testing with Dunnett's correction. Differences were considered significant at a level of $p < 0.05$.

3. Results

3.1 Inhibitory effect of nifedipine on CYP3A4 activity

The inhibitory effect of nifedipine on CYP3A4 activity is shown in Fig. 2. Nifedipine inhibited CYP3A4 enzyme activity and the 50% inhibition concentration (IC_{50}) value of nifedipine on CYP3A4 activity was 9.1 μM.

3.2 Rhodamine-123 retention assay

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, as shown in Fig. 3. The concurrent use of nifedipine enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner ranging from 10-100 μM. This result suggests that nifedipine significantly inhibit P-gp activity ($p < 0.05$).

3.3 Effect of nifedipine on the pharmacokinetics of warfarin after oral admini stration

The mean arterial plasma concentration–time profiles of warfarin after oral administration of warfarin (0.2 mg/kg) with or without nifedipine (0.5 and 2 mg/kg) are shown in Fig. 4. The relevant pharmacokinetic parameters of warfarin are also listed in Table 3. The AUC of warfarin was significantly greater (2 mg/kg, $p < 0.05$) by 13.1–36.0 %, and the C_{max} was significantly higher (2 mg/kg, $p < 0.05$) by 24.7 % after oral administration of warfarin with nifedipine. Consequently, the

relative bioavailability (R.B.) of warfarin was increased 1.13- to 1.36-fold and the absolute bioavailability (A.B.) of warfarin with nifedipine was significantly greater by 59.0–70.9 % compared to that in the control group (52.1 %). The half-life of warfarin was significantly longer (2 mg/kg, $p < 0.05$) and there was no significant change in the T_{max}.

3.4 Effect of nifedipine on the pharmacokinetics of warfarin after intravenous administration

The mean arterial plasma concentration–time profiles of warfarin after intravenous administration of warfarin (0.05 mg/kg) with or without nifedipine (0.5 or 2 mg/kg) are shown in Fig. 5. The relevant pharmacokinetic parameters of warfarin are listed in Table 4. Nifedipine had no effect on the pharmacokinetic parameters of warfarin given intravenously although it exhibited a significant effect on the bioavailability of warfarin given orally, suggesting that CYP3A4-mediated metabolism was inhibited by nifedipine, resulting in reducing intestinal or hepatic first-pass metabolism in the rats.

4. Discussion

Warfarin is an anticoagulant that has been used to prevent thromboembolism including pulmonary embolism, cardiovascular disease and stroke. Scheduled monitoring and dosage adjustment are critical to maintain efficacy and to prevent bleeding events. Warfarin has a narrow therapeutic range and its efficacy can be influenced by drug-drug interactions, drug-food interactions, genetic factors and patient characteristics [10, 15].

CYPs enzymes make a considerable contribution to the first-pass metabolism and oral bioavailability of many drugs. Moreover, induction or inhibition of CYPs may be responsible for significant drug and drug interactions [25, 26]. Modulators of P-gp can enhance or limit the permeability of a number of therapeutic agents that are considered substrates of this efflux pump protein [27]. Therefore nifedipine, a dual inhibitor against both CYP3A4 and P-gp, should have a great impact on the bioavailability of many drugs where CYP3A4 metabolism as well as P-gp mediated efflux is the major barrier to the systemic bioavailability and thus could act synergistically to limit oral bioavailability of its substrates [29].

As shown in Fig. 2, nifedipine exhibited inhibitory effect against CYP3A4-mediated metabolism with the IC_{50} values of 9.1 μM. A cell-based P-gp activity test using rhodamine-123 also showed that nifedipine did not significantly inhibit P-gp activity (Fig. 3). As nifedipine is an inhibitor of CYP3A4, concomitant use of the drug might play a role in the wide inter-individual variability in the response to drugs [30, 31]. Most calcium channel blockers (verapamil, nifedipine, diltiazem, barnidipine) also have inhibitory effect on the drug transporter P-gp, which mediates

drug's intestinal absorption [29, 32]. However, Harmsze et al [33] reported that nifedipine have poor inhibitory effects on the drug transporter P-gp.

Compared to those animals in the oral control group (warfarin without nifedipine), the AUC of warfarin was significantly greater by 13.1–36.0 %, and the C_{max} was significantly higher by 24.7 % after oral administration of warfarin with nifedipine (Table 3). Consequently, the A.B. of warfarin with nifedipine was significantly greater by 59.0-70.9 % compared to that in the control group (52.1 %). In contrast, nifedipine had no effect on any pharmacokinetic parameters of warfarin given intravenously, implying that coadministration of nifedipine could inhibit CYP3A4-mediated metabolism of warfarin, resulting in reducing intestinal or hepatic first-pass metabolism [12, 22]. These results were consistent with the results reported by Nishio et al [34].

Nifedipine significantly enhanced the oral bioavailability of warfarin, which might be mainly due to inhibition of the CYP3A-mediated metabolism of warfarin in the small intestine and /or in the liver, and inhibition of the P-gp efflux pump in the small intestine by nifedipine rather than renal elimination of warfarin.

5. Conclusion

Nifedipine significantly enhanced the oral bioavailability of warfarin, which might be mainly due to inhibition of the CYP3A-mediated metabolism of warfarin in the small intestine and /or in the liver, and inhibition of the P-gp efflux pump in the small intestine by nifedipine rather than renal elimination of warfarin. The increase in oral bioavailability of warfarin in the presence of nifedipine should be taken into consideration of potential drug interactions between warfarin and nifedipine. Furthermore, pharmacokinetic interaction between nifedipine and warfarin need to be evaluated in humans, on the basis of results, the dosage regimen of warfarin might be readjusted when used concomitantly with nifedipine in the patients.

References

[1] P. D. Henry. Comparative pharmacology of calcium antagonists: nifedipine, verapamil and diltiazem. Am J Cardiol. 1980; 46; 1047-53.

[2] E. M. Sorkin, S. P. Clissold, R. N. Brogden. Nifedipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in ischaemic heart disease, hypertension and related cardiovascular disorders. Drugs. 1985; 30; 182-7.

[3] D. G. Walter, B. S. Gruchy, A. G. Renwick, C. F. George. The first pass metabolism of nifedipine in man. Br J Clin Pharmacol. 1984; 18; 951-6.

[4] K. D. Raemsch, J. C. Sommer. Pharmacokinetics and metabolism of nifedipine. Hypertension. 1983; 5; 18-24.

[5] J. G. Kelly, K. O'Malley. Clinical pharmacokinetics of calcium antagonists: an update. Clin Pharmacokinet. 1992; 22; 416-33.

[6] F. P. Guengerich, W. R. Brian, M. Iwasaki. Oxidation of dihydropyridine calcium channel blockers and analogues by human liver cytochrome P-450 IIIA4. J Med Chem. 1991; 4; 1838-44.

[7] C. Iribarne, Y. Dréano, L. G. Bardou, J. F. Ménez, F. Berthou. Interaction of methadone with substrates of human hepatic cytochrome P450 3A4. Toxicology. 1997; 117; 13-9.

[8] V. J. Wacher, C. Y. Wu, L. Z. Benet. Overlapping substrate specificites and tissues distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Mol Carcinog. 1995; 13; 129-34.

[9] T. Endo, O. Kimura, M. Sakata. Effects of P-glycoprotein inhibitors on cadmium accumulation in cultured renal epithelial cell, LLC-PK1, and OK. Toxicol Appl Phamacol. 2002; 15; 166-71.

[10] J. Hirsh, J. E. Dalen, D. R. Anderson, L. Poller, H. Bussey, J. Ansell, D. Deykin, J. T. Brandt. Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. Chest. 1998; 114; 445-469.

[11] M. G. Scordo, V. Pengo, E. Spina, M. L. Dahl, M. Gusella, R. Padrini. Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. Clin Pharmacol Ther. 2002; 72; 702-710.

[12] R. Wallin, D. C. Sane, S. M. Hutson. Vitamin K 2,3-epoxide reductase and the vitamin K-dependent gamma-carboxylation system. Thromb Res. 2002; 108; 221-226.

[13] D. R. Mungall. Population pharmacokinetics of racemic warfarin in adult patients. J Pharmacokinet Biopharm. 1985; 13: 213-227.

[14] N. H. G. Holford. Clinical pharmacokinetics and pharmacodynamics of warfarin: understanding the dose-effect relationship. Clin Pharmacokinet. 1986; 11; 483-504.

[15] L. S. Kaminsky, Z. Y. Zhang. Human P450 metabolism of warfarin. Pharmacol Ther. 1997; 73; 67-74.

[16] U. Martinowitz, J. Rabinovich, D. Goldfarb, A. Many, H. Bank. Interaction between warfarin sodium and amiodarone. N Engl J Med. 1981; 304; 671-2.

[17] A. H. Watt, M. R. Stephens, D. C. Buss, P. A. Routledge. Amiodarone reduces plasma warfarin clearance in man. Br J Clin Pharmacol. 1985; 20; 707-9.

[18] E. Haworth, A. K. Burroughs. Disopyramide and warfarin interaction. BMJ. 1977; 2; 866-7.

[19] R. E. Kates, Y. G. Yee, E. B. Kirsten. Interaction between warfarin and propafenone in healthy volunteer subjects. Clin Pharmacol Ther. 1987; 42; 305-11.

[20] S. Kalowski, P. Kincaid-Smith. Interaction of dipyridamole with anticoagulants in the treatment of glomerulonephritis. Med J Aust. 1973; 2; 164-6.

[21] N. D. S. Bax, M. S. Lennard, S. A. H. Al-Asady, C. S. Deacon, G. T. Tucker, H. F. Woods. Inhibition of drug metabolism by beta-adrenoceptor antagonists. Drugs 1983; 25; 121-6.

[22] D. R. Abernethy, L. S. Kaminsky, T. H. Dickinson. Selective inhibition of warfarin metabolism by diltiazem in humans. J Pharmacol Exp Ther. 1991; 257; 411-415.

[23] A. M. Stoysich, B. D. Lucas, S. M. Mohiuddin, D. E. Hilleman. Further elucidation of pharmacokinetic interaction between diltiazem and warfarin. Int J Clin Pharmacol Ther. 1996; 34; 56-60.

[24] M. Zhu , K. W. Chan , L. S. Ng , Q. Chang , S. Chang , R. C. Li. Possible influences of ginseng on the pharmacokinetics and pharmacodynamics of warfarin in rats. J Pharm Pharmacol. 1999; 51; 175-180.

[25] C. L. Cummins, W. Jacobsen, L. Z. Benet. Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4. J Pharmacol Exp Ther. 2002; 300. 1036-1045.

[26] L. Z. Benet, C. L. Cummins, C. Y. Wu. Transporter-enzyme interactions: implications for predicting drug–drug interactions from in vitro data. Curr Drug Metab. 2003; 4; 393-398.

[27] R. Darvari, M. Boroujerdi. Concentration dependency of modulatory effect of amlodipine on P-glycoprotein efflux activity of doxorubicin - a comparison with tamoxifen. J Pharm Pharmacol. 2004; 56; 985-991.

[28] T. Saeki , K. Ueda , Y. Tanigawara , R. Hori , T. Komano. P-glycoprotein-

mediated transcellular transport of MDR-reversing agents. FEBS Lett. 1993; 324; 99-102.

[29] 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.

[30] J. S. Choi, Y. J. Piao, H. K. Han. Phatmacokinetics interaction between fluvastatin and diltiazem in rats. Biopharm Drug Dispos. 2006; 27; 437-441.

[31] D. H. Choi, K. S. Chang, S. P. Hong, J. S. Choi. Effect of atrovastatin on intravenous and oral pharmacokinetics of verapamil in rats. Biopharm Drug Dispos. 2008; 29; 45-50.

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

[33] A. M. Harmsze, K. Robijns, J. W. van Werkum, N. J. Breet, C. M. Hackeng, J. M. Ten Berg, H. J. Ruven, O. H. Klungel, A. de Boer, V. H. Deneer. The use of amlodipine, but not of P-glycoprotein inhibiting calcium channel blockers is associated with clopidogrel poor-response. Thromb Haemost. 2010; 103; 920-925.

[34] S. Nishio, H. Watanabe, K. Kosuge, S. Uchida, H. Hayashi, K. Ohashi. Interaction between amlodipine and simvastatin in patients with hypercholesterolemia and hypertension. Hypertens Res. 2005; 28; 223-227.

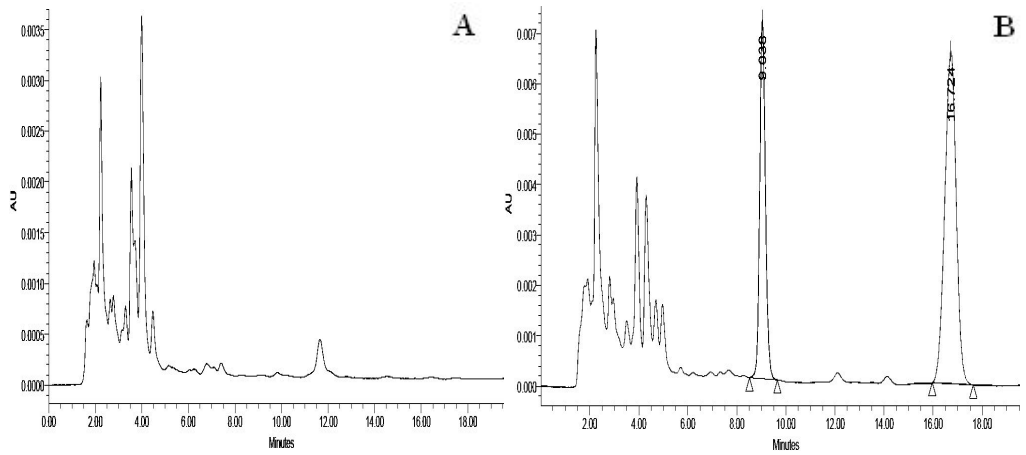

Figure 1. HPLC chromatograms of the rat's blank plasma (A), and the plasma spiked with warfarin (16.7 min) and 7-ethoxycoumarin (internal standard; 9.0 min) (B).

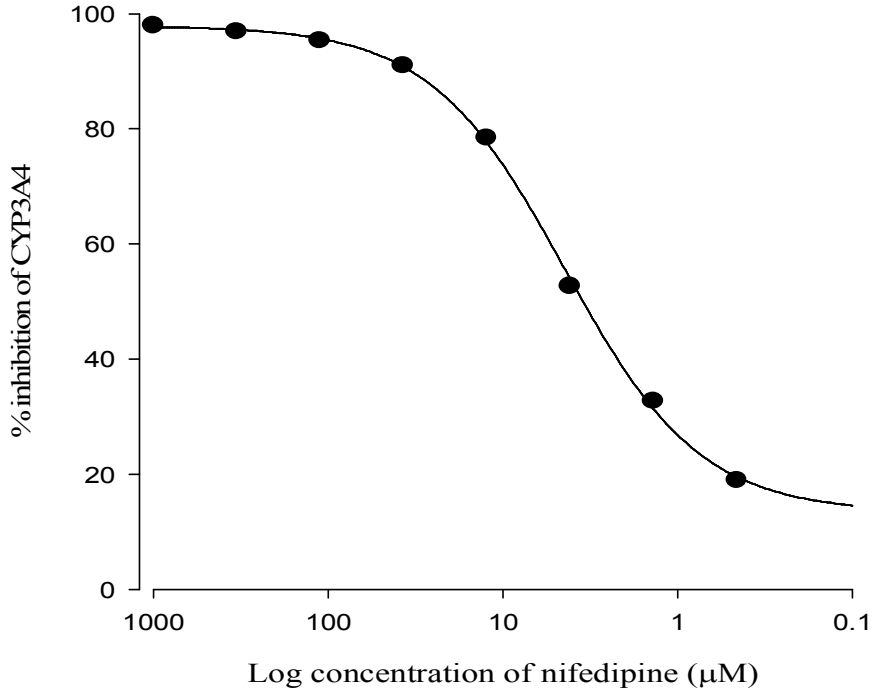

Figure 2. Inhibitory effect of nifedipine on CYP3A4 activity. All experiments were performed in duplicate, and results were expressed as the percent of inhibition (IC_{50}: 9.1 μM).

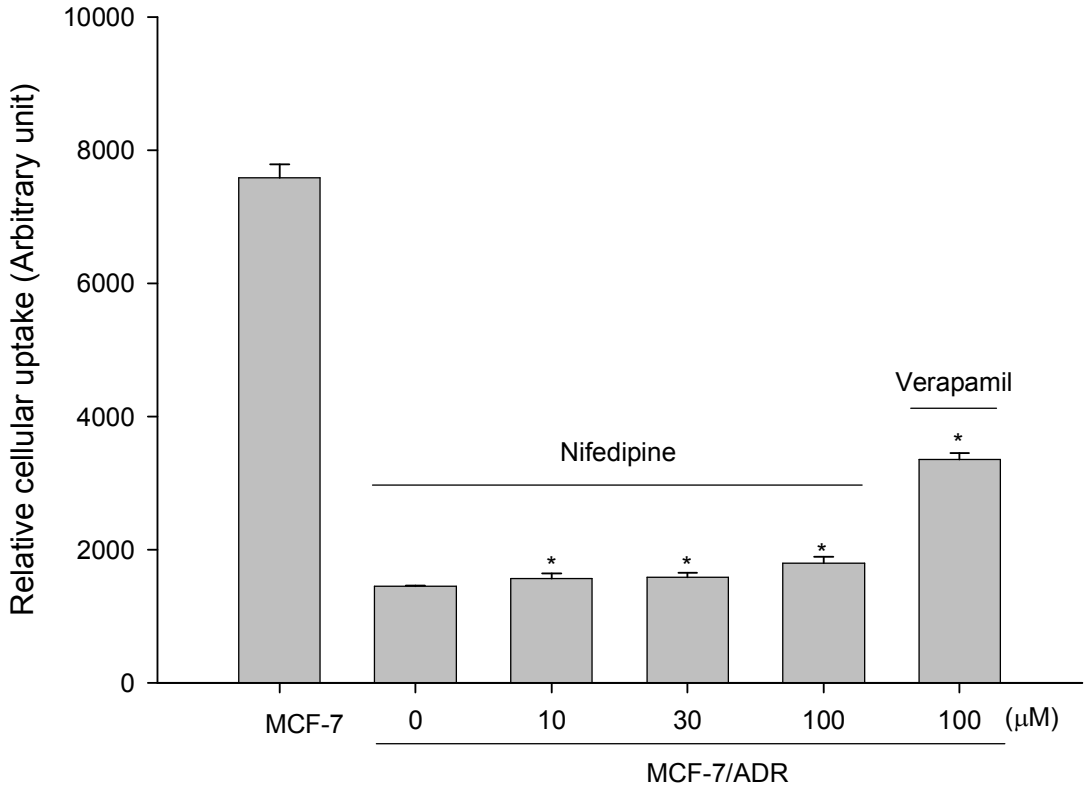

Figure 3. Rhodamine-123 retention. MCF-7/ADR cells were preincubated with nifedipine for 30 min, and after incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min. The rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. The values were divided by total protein contents of each sample. Verapamil (100 μM) was used as a positive control. Data represents mean ± SD of 6 separate samples.

* $p < 0.05$, significant difference compared to control group.

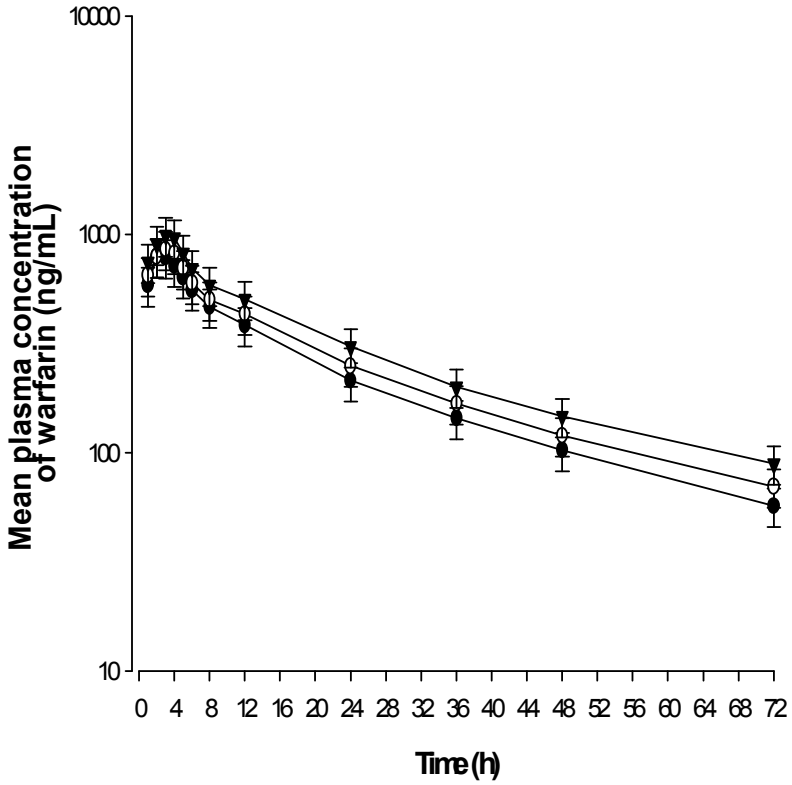

Figure 4. Mean arterial plasma concentration–time profiles of warfarin after oral administration of warfarin (0.2 mg/kg) without (●) or with 0.5 mg/kg (○) and 2 mg/kg (▼) of nifedipine in rats. Bars represent the standard deviation (n = 6).

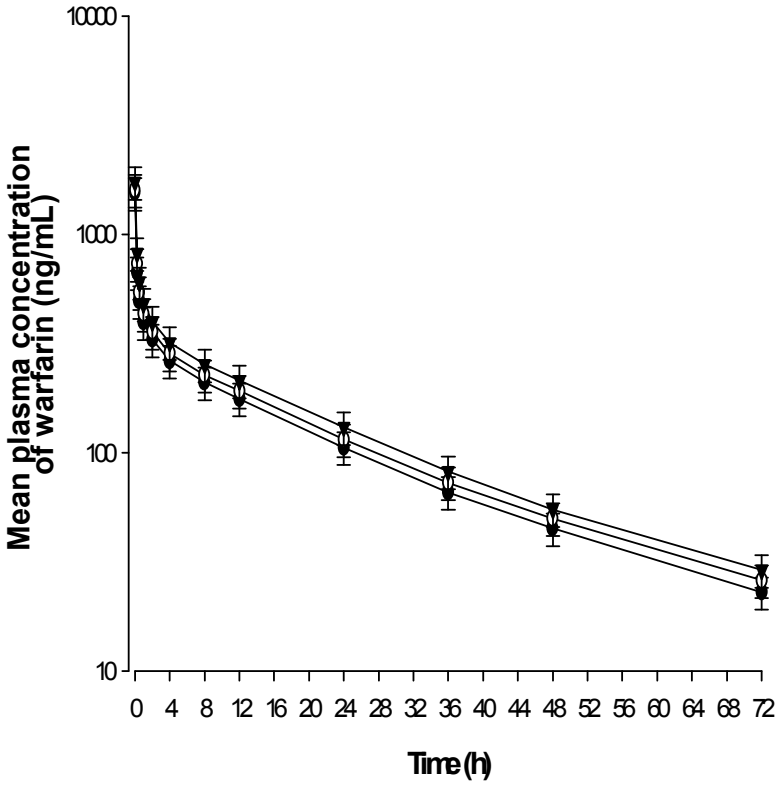

Figure 5. Mean arterial plasma concentration–time profiles of warfarin after intravenous administration of warfarin (0.05mg/kg) without (●) or with 0.5 mg/kg (○) and 2 mg/kg (▼) of nifedipine in rats. Bars represent the standard deviation (*n* = 6).

Table 1. Mean plasma concentration of warfarin after oral administration of warfarin (0.2 mg/kg) with or without nifedipine (0.5 and 2 mg/kg) in rats (Mean ± SD, $n = 6$).

Time (h)	Control	With Nifedipine	
	without nifedifine	0.5 mg/kg	2 mg/kg
1	585 ± 111.2	651 ± 123.7	749 ± 142.3
2	794 ± 150.9	792 ± 150.5	903 ± 171.6
3	786 ± 149.3	854 ± 162.3	990 ± 188.1
4	717 ± 136.2	821 ± 160	965 ± 183.4
5	636 ± 120.8	701 ± 133.2	822 ± 156.2
6	558 ± 106	600 ± 114	701 ± 133.2
8	466 ± 88.5	503 ± 95.6	586 ± 111.3
12	385 ± 73.2	432 ± 82.1	505 ± 96
24	214 ± 40.7	250 ± 47.5	306 ± 58.1
36	144 ± 27.4	168 ± 31.9	200 ± 38
48	103 ± 19.6	120 ± 22.8	147 ± 27.9
72	57 ± 10.8	70 ± 13.3	89 ± 16.9

Table 2. Mean plasma concentration of warfarin after intravenous administration of warfarin (0.05 mg/kg) with or without nifedipine (0.5 and 2 mg/kg) in rats (Mean ± SD, n = 6).

Time (h)	Control without nifedifine	With Nifedipine	
		0.5 mg/kg	2 mg/kg
0	1548 ± 294.1	1599 ± 303.8	1740 ± 330.6
0.25	671 ± 127.5	733 ± 139.3	820 ± 155.8
0.5	496 ± 94.2	542 ± 103	604 ± 114.8
1	396 ± 75.2	432 ± 82.1	481 ± 91.4
2	330 ± 62.7	358 ± 68	399 ± 75.8
4	264 ± 50.2	285 ± 54.2	320 ± 60.8
8	210 ± 39.9	227 ± 43.1	254 ± 48.3
12	177 ± 33.6	192 ± 36.5	214 ± 40.7
24	106 ± 20.1	115 ± 21.9	131 ± 24.7
36	66 ± 12.5	73 ± 13.9	82 ± 15.6
48	45 ± 8.6	50 ± 9.5	55 ± 10.5
72	23 ± 4.4	26 ± 4.9	29 ± 5.5

Table 3. Mean pharmacokinetic parameters of warfarin after oral administration of warfarin (0.2 mg/kg) with or without nifedipine (0.5 and 2 mg/kg) in rats (Mean ± SD, $n = 6$).

Parameters	Control	With nifedipine	
	Without nifedipine	0.5 mg/kg	2 mg/kg
AUC (ng·h/mL)	15462 ± 3090	17493 ± 3491	21026 ± 4201*
C_{max} (ng/mL)	794 ± 152	854 ± 189	990 ± 198*
T_{max} (h)	2.0	3.0	3.0
$t_{1/2}$ (h)	27.0 ± 3.5	28.8 ± 3.8	31.4 ± 4.0*
CL/F (ml/min/kg)	11.3 ± 2.8	9.8 ± 2.6	8.1 ± 2.0
A.B. (%)	52.1 ± 9.8	59.0 ± 12.5	70.9 ± 14.6*
R.B. (%)	100	113	136

* $p < 0.05$, ** $p < 0.01$, significant difference compared to control group.

AUC, area under the plasma concentration–time curve from 0 h to time infinity; C_{max}, peak plasma concentration; T_{max}, time to reach peak concentration; $t_{1/2}$, terminal half-life; CL/F, total body clearance; A.B., absolute bioavailability; R.B., relative bioavailability.

Table 4. Mean pharmacokinetic parameters of warfarin after intravenous administration of warfarin (0.05 mg/kg) with or without nifedipine (0.5 and 2 mg/kg) in rats (Mean ± SD, n = 6).

Parameters	Control	With nifedipine	
	Without nifedipine	0.5 mg/kg	2 mg/kg
AUC (ng·h/mL)	7418 ± 1459	8089 ± 1508	8976 ± 1751
CL_t (mL/min/kg)	6.2 ± 1.4	5.5 ± 1.3	5.0 ± 1.3
$t_{1/2}$ (h)	23.8 ± 4.3	24.2 ± 4.5	24.5 ± 4.6
R.B. (%)	100	109	121

AUC, area under the plasma concentration-time curve from time 0 to infinity; CL_t, total body clearance; $t_{1/2}$, terminal half-life; R.B., relative bioavailability.

Acknowledgements

지난 2 년간의 대학원 생활에서 여러가지로 부족한 저를 이끌어주시고, 힘들 때마다 격려와 충고를 아끼지 않으신 지도 교수님, 최준식 교수님께 무한한 감사를 드리며, 부족한 논문을 심사하여 향상 시켜주신 최후균 교수님과 강건욱 교수님께 감사를 드립니다.

또한 항상 뒤에서 지켜봐주시는 최인 약제부장님께도 감사의 말씀을 전하고 싶습니다.

저의 논문을 실험하는대 도와주신 약제학교실 이성 선생님께 깊은 감사의 말씀 드립니다.

마지막으로 어려운 상황 속에서 항상 제 버팀목이 되어주고 힘을 실어준 사랑하는 양가 부모님과 남편과 아들·딸에게 감사하다고 전해드리고 싶습니다.

저작물 이용 허락서

학 과	약학과	학 번	20097348	과 정	석사
성 명	한 글 : 박선희	한 문 :朴 宣 姬		영 문 : Sun-Hee Park	
주 소	광주광역시 동구 서석동 조선대학교 병원 약제부				
연락처	E-mail : juliet0329@hanmail.net				
논문제목	한글: 흰쥐에서 니페디핀과 왈파린과의 약물동태학적 상호작용 영문: Pharmacokinetic Interaction between Nifedipine and Warfarin 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 ( )

2011 년 2 월

저작자: 박선희 (서명 또는 인)

조선대학교 총장 귀하