2011 년 2 월

석사학위논문

PHARMACOKINETIC INTERACTION BETWEEN PRAVASTATIN AND WARFARIN IN RATS

조선대학교 대학원

의 학 과

양 준 승

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흰쥐에서 프라바스타틴과 와파린의 약물동태학적 상호작용

2011 년 2 월 25 일

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이 논문을 의학석사학위신청 논문으로 제출함.

2010 년 10 월

조선대학교 대학원

의학과

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2010 년 11 월

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Pharmacokinetic Interaction between Pravastatin and Warfarin in Rats

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The aim of this study was to investigate the effect of pravastatin 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 pravastatin (0.1 or 0.4 mg/kg) in rats. The effect of pravastatin on the P-glycoprotein (P-gp) as well as cytochrome P450 (CYP) 3A4 activity was also evaluated. Pravastatin 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 pravastatin), the area under the plasma concentration–time curve (AUC) of warfarin was significantly greater (0.1 mg/kg, $P<0.05$; 0.4 mg/kg, $P<0.01$) by 26.5–53.5 %, and the peak plasma concentration (C_{max}) was significantly higher (0.4 mg/kg, $P<0.05$) by 26.2 % after oral administration of warfarin with pravastatin, respectively. Consequently, the relative bioavailability of warfarin increased by 1.26- to 1.53-fold and the absolute

bioavailability of warfarin with pravastatin was significantly greater by 61.7–72.5 % compared to that in the control group (47.4 %). In contrast, pravastatin 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 pravastatin.

Key words: Warfarin, Pravastatin, CYP3A, P-gp, Pharmacokinetics, Bioavailability, Rats

국 문 초 록

흰쥐에서 프라바스타틴과 와파린의 약물동태학적 상호작용

양 준 승
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본 연구의 목적은 쥐에 경구 또는 정맥주사로 warfarin 을 투여한 후에 warfarin 의 약물 동태학적인 pravastatin 의 효과를 조사하는 것이었다. 쥐에 pravastatin(0.1 or 0.4 mg/Kg)을 경구로 투여한 군과 투여하지 않은 군으로 나누어 Warfarin 을 경구(0.2 mg/Kg) 또는 정맥주사로(0.05 mg/Kg) 투여했다. Cytochrome P450(CYP) 3A4 뿐 아니라 P-glycoprotein(P-gp)활성도에 대한 pravastatin 의 효과도 검사되었다. Pravastatin 은 9.1 μM 의 반수 치사농도에서 CYP3A4 효소 활성이 억제 되었다.

Pravastatin 을 투여하지 않은 동물 대조군에 비하여 pravastatin 과 warfarin 을 병용 투여군에서 warfarin 의 혈장농도곡선하면적(AUC)은 26.5-53.5%(0.1 mg/kg, $P<0.05$; 0.4 mg/kg, $P<0.01$)으로 의미 있게 증가되었으며 최고혈중농도도 26.2%(0.4 mg/kg, $P<0.05$)로 각각 유의성 있게 증가 되었다. 결과적으로, 대조군에 비하여 pravastatin 과 warfarin 을 병용 투여한

군의 warfarin 의 상대적인 생체 이용 효율은 1.26 에서 1.53 배였으며 절대적인 생체 이용률은 대조군(47.4%)에 비하여 61.7-72.5%으로 의미있게 높았다. 반대로 pravastatin 은 정맥으로 투여한 warfarin 의 어떤 척도에도 약물 동태학적으로 영향을 보이지 않았다. 강화된 warfarin 의 경구 생체 이용률은 pravastatin 의 일차 통과 대사가 감소된 결과로 제거되는 것보다 장관이나 간의 CYP 3A4 관련된 대사의 억제로 기인한 것으로 사료된다.

Introduction

Pravastatin, one of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) widely used in the management of hypercholesterolaemia [1]. Pravastatin is rapidly but incompletely absorbed from the gastrointestinal tract and undergoes extensive first-pass metabolism in the liver, its primary site of action [2]. 3″-hydroxy pravastatin and 3′α, 5′β-dihydroxy-pravastatin are the major metabolites of pravastatin [1]. Cytochrome P450 (CYP) 3A is mainly responsible for 3″-hydroxy pravastatin formation, whereas members of the CYP enzymes other than the CYP3A subfamily are also involved in the formation of 3′α, 5′β-dihydroxy-pravastatin [3-5].

There is no clinically important pharmacokinetic interaction of pravastatin with a number of common CYP3A inhibitors. Itraconazole, diltiazem and grapefruit juice have all no statistically significant effect on pharmacokinetics of pravastatin [6-8]. The contribution of CYP-dependent biotransformation to pravastatin elimination is minor. Pharmacokinetics interaction of pravastatin with other drugs are rare compared with those of other statins, this may be due to the dual routes of elimination and low plasma protein binding of pravastatin. Pravastatin seems to be more favourable in the management of hypercholesterolaemia compared with the other statins.

Pravastatin does not undergo significant cytochrome P-450 (CYP)-mediated biotransformation. The organic anion transporting polypeptide 1B1 (OATP1B1),

and multidrug resistance-associated protein 2 (MRP2), are thought to be the major transporters involved in the pharmacokinetics of pravastatin in humans [9]. Kato et al reported that cyclosporine A transport could competited inhibited by pravastatin via MRP 2 [10].

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 [11]. 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 [12,13]. 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 [14]. Warfarin is metabolized by CYPs and is converted to inactive metabolites through selective hydroxylation [15]. R-warfarin is metabolized primarily by CYP3A4 to 10-hydroxywarfarin, by CYP1A2 to 6- and 8-hydroxywarfarin. 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].

However, there have been no reports regarding the effects of pravastatin on the bioavailability or pharmacokinetics of warfarin in rats. Moreover, pravastatin 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 pravastatin on the pharmacokinetics of warfarin after oral and intravenous administration in rats.

Materials and Methods

Materials

Warfarin, pravastatin 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).

Animal studies

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 24 h 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.

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 °C for 30 min; 1.0 mL/kg) without (control) or with 0.1 or 0.4 mg/kg of oral pravastatin, and an intravenous group (0.05 mg/kg of warfarin, dissolved in 0.9 % NaCl-injectable solution; homogenized at 36 °C for 30 min; 0.3 mL /kg) without (control) or with 0.1 or 0.4 mg/kg of oral pravastatin. Warfarin was administered orally using a gastric gavage tube, and pravastatin 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.

HPLC assay

The plasma concentrations of warfarin were determined by a HPLC assay method reported by Zhu et al. (1999). 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 ^{o}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. The detection limit of warfarin in rat's plasma was 5 ng/mL. The coefficients of variation for warfarin were below 12.8 %.

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.

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 pravastatin (1, 3 or 10 μM) or warfarin (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.

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 $AUC_{with\ pravastatin}/AUC_{control} \times 100$.

Statistical analysis

All data were expressed with their standard deviation (mean ± S.D.). 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 $p<0.05$.

Results

Inhibitory effect of pravastatin on CYP3A4 activity

The inhibitory effect of pravastatin on CYP3A4 activity is shown in Fig. 1. Pravastatin inhibited CYP3A4 enzyme activity and the 50 % inhibition concentration (IC_{50}) value of pravastatin on CYP3A4 activity was 9.1 μM.

Rhodamine-123 retention assay

Accumulation of rhodamine-123, a P-gp substrate, was not raised in MCF-7/ADR cells overexpressing P-gp compared to that in MCF-7 cells lacking P-gp, as shown in Fig. 2. The concurrent use of pravastatin did not enhance the cellular uptake of rhodamine-123 in a concentration-dependent manner ranging from 1-10 μM . This result suggests that pravastatin did not significantly inhibit P-gp activity.

Effect of pravastatin on the pharmacokinetics of warfarin after oral administration

The mean arterial plasma concentration–time profiles of warfarin after oral administration of warfarin (0.2 mg/kg) with or without pravastatin (0.1 and 0.4 mg/kg) are shown in Fig. 3. The relevant pharmacokinetic parameters of warfarin are also listed in Table 1. The AUC of warfarin was significantly greater (0.1 mg/kg, $P<0.05$; 0.4 mg/kg, $P<0.01$) by 26.5–53.5 %, and the C_{max} was significantly higher (0.4 mg/kg, $P< 0.05$) by 26.2 % after oral administration of warfarin with

pravastatin. Consequently, the relative bioavailability of warfarin was increased 1.26- to 1.53-fold and the absolute bioavailability of warfarin with pravastatin was significantly greater by 61.7–72.5 % compared to that in the control group (47.4 %). The half-life of warfarin was significantly longer (0.4 mg/kg, $P < 0.05$) and there was no significant change in the T_{max}.

Effect of pravastatin 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 pravastatin (0.1 or 0.4 mg/kg) are shown in Fig. 4. The relevant pharmacokinetic parameters of warfarin are listed in Table 2. Pravastatin 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 pravastatin, resulting in reducing intestinal or hepatic first-pass metabolism.

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 [11, 16].

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 [17, 18]. 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 [19]. Therefore pravastatin, 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 [20, 21].

As shown in Fig. 1, pravastatin 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 pravastatin did not significantly inhibit P-gp activity (Fig. 2).

Compared to those animals in the oral control group (warfarin without pravastatin), the AUC of warfarin was significantly greater by 26.5–53.5 %, and the C_{max} was significantly higher by 26.2 % after oral administration of warfarin with pravastatin (Table 1). Consequently, the absolute bioavailability of warfarin with pravastatin was significantly greater by 61.7–72.5 % compared to that in the control group (47.4%). In contrast, pravastatin had no effect on any pharmacokinetic parameters of warfarin given intravenously, implying that coadministration of pravastatin could inhibit CYP3A4-mediated metabolism of warfarin, resulting in reducing intestinal or hepatic first-pass metabolism [22].

Conclusion

The enhanced oral bioavailability of warfarin by pravastatin suggests that CYP3A4-mediated metabolism were inhibited rather than P-gp-mediated efflux or renal elimination of warfarin. In further clinical studies, the dosage regimen of warfarin might be readjusted when used concomitantly with pravastatin in the patients.

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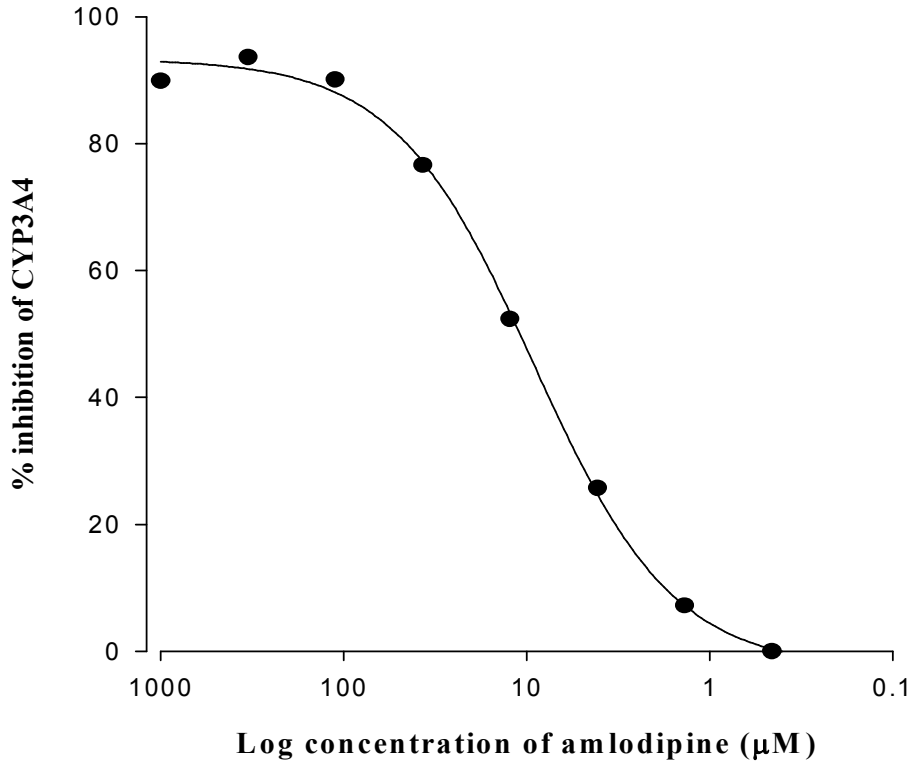

Figure 1. Inhibitory effect of pravastatin on CYP3A4 activity. All experiments were performed in duplicate, and results were expressed as the percent of inhibition (IC_{50}: 9.1 μM).

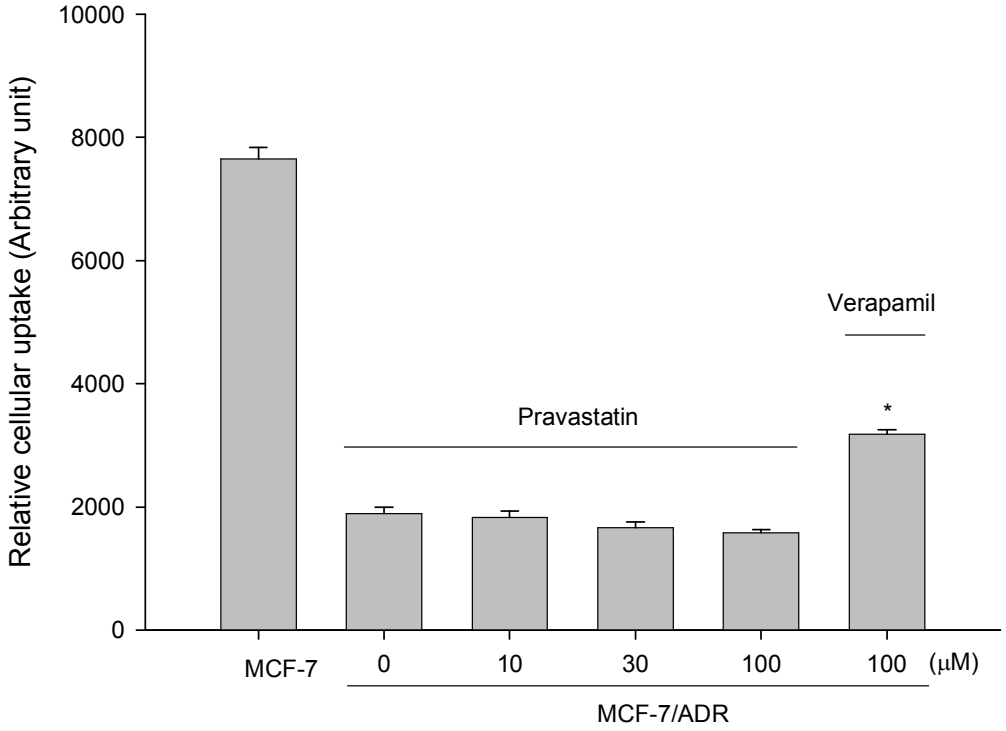

Figure 2. Rhodamine-123 retention. MCF-7/ADR cells were preincubated with pravastatin for 30 min, and after incubation of MCF-7/ADR cells with 20 μM R-123 for 90 min. The rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. 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.

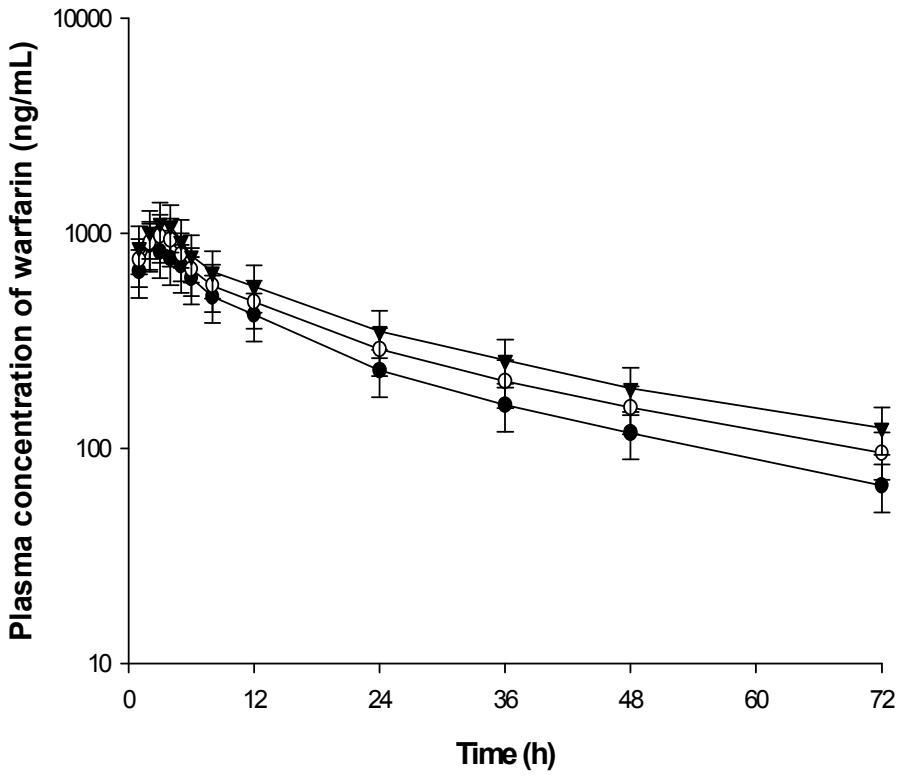

Figure 3. Mean arterial plasma concentration–time profiles of warfarin after oral administration of warfarin (0.2 mg/kg) without (●) or with 0.1 mg/kg (○) and 0.4 mg/kg (▼) of pravastatin in rats. Bars represent the standard deviation (n = 6).

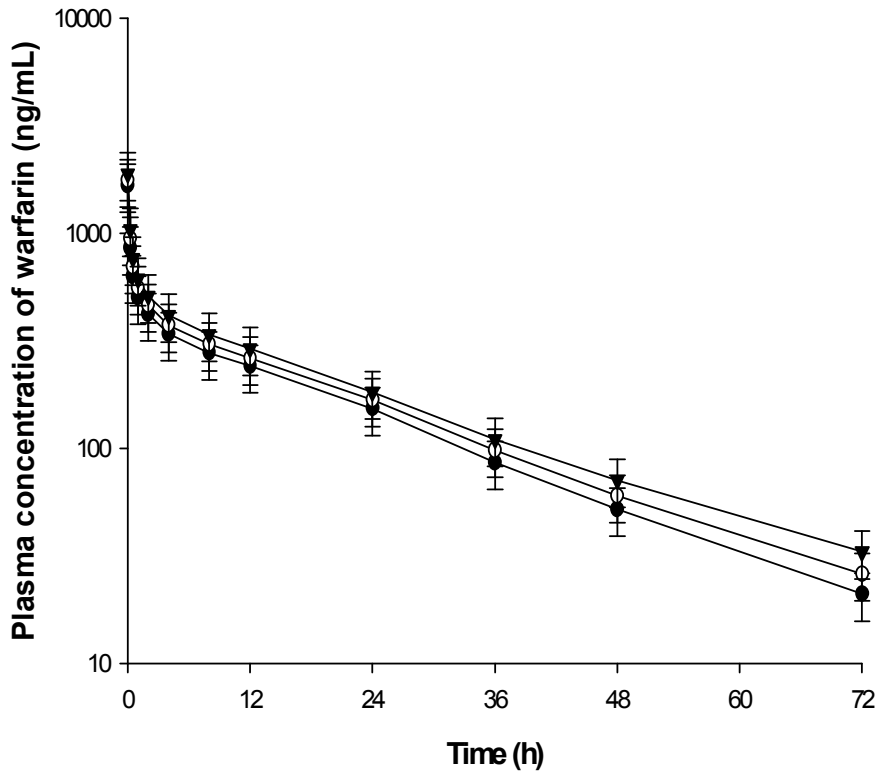

Figure 4. Mean arterial plasma concentration–time profiles of warfarin after intravenous administration of warfarin (0.05mg/kg) without (●) or with 0.1 mg/kg (○) and 0.4 mg/kg (▼) of pravastatin in rats. Bars represent the standard deviation (n = 6).

Table 1. Mean pharmacokinetic parameters of warfarin after oral administration of warfarin (0.2 mg/kg) with or without pravastatin (0.1 and 0.4 mg/kg) in rats

Parameters	Control	With pravastatin	
	Without pravastatin	0.1 mg/kg	0.4 mg/kg
AUC (ng·h/mL)	19810 ± 3358	25054 ±4510*	30417 ±6689**
C_{max} (ng/mL)	882 ± 152	972 ± 211	1113 ±202*
T_{max} (h)	2.0	3.0	3.0
$t_{1/2}$ (h)	27.6 ± 3.1	31.6 ± 5.4	33.0 ± 4.6*
CL/F(ml/min/kg)	10.1 ± 2.9	7.9 ± 2.3	6.8 ± 2.0
A.B. (%)	47.4 ± 8.8	61.7 ± 12.5*	72.5 ± 15.1**
R.B. (%)	100	126	153

Mean ± S.D. (n=6), * $P < 0.05$, ** $P < 0.01$, significant difference compared to controls, 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 2. Mean pharmacokinetic parameters of warfarin after intravenous administration of warfarin (0.05 mg/kg) with or without pravastatin (0.1 and 0.4 mg/kg) in rats

Parameters	Control Without pravastatin	With pravastatin	
		0.1 mg/kg	0.4 mg/kg
AUC(ng·h/mL)	10149 ± 2230	11275 ± 2254	12845 ± 2565
CL(mL/min/kg)	9.8 ± 2.7	8.9 ± 2.4	7.6 ± 2.0
$t_{1/2}$(h)	18.1 ± 3.7	18.9 ± 3.9	20.2 ± 4.2

Mean ± S.D. (n=6), AUC: area under the plasma concentration-time curve from time 0 to infinity, CL: total body clearance, $t_{1/2}$: terminal half-life, R.B. (%): relative bioavailability.

Acknowledgements

새로운 배움과 학위를 취득하며 무언가를 이루었다는 성취감에 마음이 뿌듯하며 이 모든 것을 이루는데 밑거름이 되게 하신 모든 분들께 감사의 말을 전하고자 합니다.

좋은 연구 테마를 주시고 물심양면으로 격려와 조언으로 학위의 꿈을 주신 최동현 교수님께 머리 숙여 감사를 드립니다.

항상 격려와 조언을 아끼지 않으신 내과 김현숙 교수님, 이준 교수님께 깊은 감사를 드립니다.

학위논문을 실험할 때 도와주신 약학대학 최준식 교수님과 이 성 선생님께도 진심으로 감사를 드립니다.

항상 기도해 주시고 염려해 주신 양가 부모님께 한없는 감사를 드립니다.

그리고 항상 저의 옆에서 고생을 아끼지 않은 사랑하는 아내와 아들에게 고마움을 전하고 싶습니다.

석사학위를 가진 의사로서 이웃과 환자들에게 봉사하고, 더욱 더 매진하여 세상의 빛과 소금이 되어 하나님께 영광을 돌리는 의사가 되고자 노력 하겠습니다.

저작물 이용 허락서

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논문제목	한글: 흰쥐에서 프라바스타틴과 와파린의 약물동태학적 상호작용. 영문: Pharmacokinetic interaction between pravastatin 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 ( )

2010 년 12 월

저작자: 양준승 (서명 또는 인)

조선대학교 총장 귀하