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2010 년 8 월

석사학위논문

Effects of silibinin, an antioxidant, on the bioavailability of Losartan in Rats

조선대학교 대학원

식품의약학과

이 재 성

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생체이용률에 미치는 영향

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이 논문을 약학석사학위신청 논문으로 제출함.

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Effects of silibinin, an antioxidant, on the bioavailability of Losatan in Rats

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Abstract

The present study was to investigate the effect of silibinin, an antioxidant, on the bioavailability and pharmacokinetics of losartan and its active metabolite, EXP-3174, in rats. The pharmacokinetic parameters of losartan and EXP-3174 in rats were determined after oral administration of losartan (9 mg/kg) in the presence or absence of silibinin (0.3, 1.5 and 6 mg/kg). The effect of silibinin on the P-glycoprotein (P-gp), CYP 3A4 and CYP2C9 activity was also evaluated. Silibinin inhibited CYP3A4 and CYP2C9 enzyme activity in a concentration-dependent manner with 50% inhibition concentration (IC50) of 1.97 and 5.25 μM, respectively.

In addition, silibinin significantly enhanced the cellular accumulation of rhodamine 123 in MCF-7/ADR cells overexpressing P-gp. The pharmacokinetic parameters of losartan were significantly altered by silibinin compared with those in the control group. The presence of silibinin significantly ($p < 0.05$, 1.5 mg/kg; $p < 0.01$, 6 mg/kg) increased the area under the plasma concentration–time curve (AUC0–∞) of losartan by 40.8–68.4% and the peak plasma concentration (Cmax) of losartan by 40.6–69.3%. Consequently, the absolute bioavailability (A.B.) of losartan in the presence of silibinin was 35.8–42.8%, which was enhanced significantly ($p < 0.05$) compared with that in the oral control group (25.4%). The relative bioavailability (R.B.) of losartan was increased by 1.41- to 1.68-fold over that in the control group. However, there were no significant changes in the time to reach the peak plasma concentration (Tmax) and the terminal half-life (t1/2) of losartan in the presence of silibinin. Silibinin (6 mg/kg) significantly increased the AUC (28.6%) of EXP-3174 compared with that in the control group. The metabolite-parent AUC ratio (M.R.) was significantly ($p < 0.05$) decreased by 32.1–35.8 % in the presence of silibinin (1.5 and 6 mg/kg) compared to that in the control group, implying that coadministration of silibinin could inhibit the CYP3A and CYP2C9-mediated metabolism of losartan in the small intestine or in the liver and the P-gp efflux pump in the small intestine. In conclusion, silibinin significantly enhanced the oral bioavailability of losartan, suggesting that concurrent use of silibinin and losartan should be monitored closely for potential drug interactions.

Key words: losartan, EXP-3174, silibinin, bioavailability, pharmacokinetics, P-gp, CYP3A4, CYP2C9, rat

국 문 초 록

흰쥐에서 항산화제인 실리비닌이 로살탄의 생체이용률에 미치는 영향

이 재 성

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본 연구는 흰쥐에서 항산화제인 실리비닌이 고혈압치료제인 로살탄의 생체이용률과 약물동태에 미치는 영향을 검토하였다. 로살탄과 대사체인 EXP-3174 의 약물동태는 실리비닌(0.3, 1.5 및 6mg/kg)과 로살탄(9mg/kg) 경구투여 하여 본연구를 실시하였다. 실리비닌이 p-glycoprotein(p-gp), CYP3A4 및 CYP2C9 의 활성에 미치는 영향도 평가하였다. 실리비닌은 p-gp 를 과량유입시킨 MCF-7/ADR 세포내에서 rhodamine 123 세포의 축적을 증가 시켰다. 실리비닌은 로살탄의 혈장농도곡선하면적(AUC)을 40.6~68.4%로 증가 시켰으며, 최대 최고혈장농도는 40~69.3%으로 증가시켰다. 따라서 실리비닌은 로살탄의 절대적생체이용율(AB)는 35.8~42.8%로 증가시켰으며, 상대적생체이용율(RB)은 대조군에 비해 1.41~1.68 배 증가시켰다. 그러나 실리비닌은 Tmax 에 도달하는 시간과 반감기에는 거의 영향을 미치지 못하였다. 실리비닌(6mg/kg)은 EXP-3174 의 AUC(28.6%)를 증가시켰다. 실리비닌(1.5, 6mg/kg)은 로살탄의 대사률(metabolite-parent AUC ratio; M.R)를 32.1~35.8%로 감소시켰다. 이는 실리비닌을 로살탄과 병용경구 투여시 소장과 간장에서 CYP3A4 과 CYP22CP 활성억제와 소장에서 P-헤 활성을 억제시킨 것으로 사료된다.

결과적으로 실리비닌은 로살탄의 생체이용률 증가시킨다는 점에서 실리비닌과 로살탄의 병용투여는 약물상호작용의 초래를 면밀히 검토되어야 한다고 사료된다.

1. Introduction

Losartan potassium (DuP 753 or MK-954), an angiotensin II receptor antagonist, is the first of a new class of agents introduced for the treatment of hypertension [12, 25]. Two angiotensin receptor subtypes, angiotensin receptor-1 (AT1) and angiotensin receptor-2 (AT2), have been proposed on the basis of ligand-binding studies [10]. 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 [19]. After oral administration of losartan, about 5% of the dose is excreted unchanged in the urine and about 10% 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) [20]. Soldner et al. have suggested that losartan is a substrate of P-glycoprotein (P-gp) [30]. *In vitro* and *in vivo* studies have demonstrated that losartan is metabolized by CYP3A4 and CYP2C9 [15, 21, 22, 31, 32, 36]. Considering that P-gp is co-localized with CYP3A4 in small intestine, P-gp and CYP3A4 may act synergistically in presystemic drug metabolism and lead to prolonged exposure of P-gp substrates to CYP3A4, resulting in the limited absorption of drugs [7, 8, 11, 33, 34].

Flavonoids are phytochemicals that are produced in high quantities by various plants [5]. These compounds exhibit a wide range of beneficial biological activities including antioxidative, radical-scavenging, anti-atherosclerotic, antitumor and antiviral effects [24]. Silymarin, a flavonoid complex, is extracted from seeds of the milk thistle (*Silybum marianum* L.), which is a medicinal plant widely used in traditional European medicine [23]. Silymarin has strong antioxidant activity [38] and exhibits cytoprotective, anti-inflammatory, and anticarcinogenic effects [14]. Silibinin is the major and most active component in silymarin, comprising 60–70%

of silymarin [17]. Kosina et al. [17] have reported that silibinin inhibits human CYP 1A2 and 3A4, while Zuber et al. [39] found that silibinin inhibits human CYP 2D6, 2C9 and 3A4. Thus, the inhibitory effects of silibinin against human CYP enzymes remain somewhat controversial. Silibinin is an inhibitor of P-gp in the KB/MDR cell line [6], but the inhibitory effect of silibinin against P-gp is ambiguous. Therefore, we re-evaluated the inhibition of CYP enzyme activity and P-gp activity by silibinin using the CYP inhibition assay and the rhodamine-123 retention assay in P-gp-overexpressing MCF-7/ADR cells.

Given that the bioavailability of losartan is mainly affected by CYP3A4 or CYP2C9 and P-gp during first-pass metabolism, inhibitor of CYP3A4 and CYP2C9 and P-gp such as silibinin could be expected to improve the bioavailability of losartan. Silibinin and losartan sometimes might be prescribed as a combination therapy. However, the possible effects of silibinin on the bioavailability and pharmacokinetics of losartan and its active metabolite, EXP-3174, in rats have not been reported *in vivo*.

Therefore, the aim of this study was to investigate the effects of silibinin on the bioavailability or pharmacokinetics of losartan and its active metabolite, EXP-3174, in rats.

2. Materials and methods

2.1. Materials

Losartan, its metabolite EXP-3174 and the internal standard L-158.809 were obtained from Merck Co. (NJ, U.S.A.). Silibinin was 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. Animal Studies

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 experimental protocols were approved by the Animal Care Committee of Chosun University. Male Sprague-Dawley rats (280-300 g) were purchased from Dae Han Laboratory Animal Research and Co. (Eumsung, Republic of Korea), and had free access to normal standard chow diet (Superfeed Company, Wonju, Republic of Korea) and tap water throughout the experiment, the animals were housed, four or five per cage, in laminar flow cages maintained at 22 ± 2 °C, 50-60% relative humidity, under a 12 h light-dark cycle. The animals were kept in these facilities for at least one week before the experiment.

2.3. Drug administration

The rats were divided into five groups (n = 6, each group): an oral control group (9 mg/kg of losartan dissolved in distilled water) without (control) or with oral administration of silibinin at a dose of 0.3, 1.5 or 6 mg/kg (in distilled water) and 3

mg/kg of losartan dissolved in 0.9% NaCl solution for intravenous administration. The rats were fasted for at least 24 h prior to the beginning of the experiments. Each animal was anaesthetized with ether and the right femoral artery was cannulated with a polyethylene tube (SP45, I.D. 0.58 mm, O.D. 0.96 mm; Natsume Seisakusho Co. Ltd, Tokyo, Japan) for blood sampling. Oral losartan was administered intragastrically using a feeding tube, and silibinin was administered intragastrically 30 min before the oral administration of losartan. Losartan for iv administration was injected through the femoral vein within 0.5 min. A 0.4-ml aliquot of blood was collected into heparinized tubes from the femoral artery at 0 (to serve as control), 0.1 (only for iv group), 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after losartan administration. The blood samples were centrifuged at 16810 × g for 5 min, and the plasma sample (0.2 ml) was stored at –40 °C until losartan and EXP-3174 was analyzed by HPLC.

2.4. Method and assay

2.4.1 HPLC Assay

The plasma concentrations of losartan were determined by the HPLC assay modified from Zarghi et al [37]. Briefly, a 50-µl aliquot of L-158.809 (0.2 µg/ml dissolved in methanol; an internal standard) and a 0.5-ml aliquot of acetonitrile were added to a 0.2-ml aliquot of the plasma in a 2.0-ml polypropylene microtube. The mixture was then stirred for 5 min and centrifuged (16810 × g, 10 min). A 0.4-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 150 µl aliquot of the mobile phase and centrifuged (16810 × g, 5 min). A 70-µl aliquot of the water layer was injected into the HPLC system. The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu Co., Japan), a UV-Vis detector (Model SPD-10A), a system controller (Model SCL-10A), degasser

(Model DGU-12A) and an autoinjector (SIL-10AD). The UV detector was set to 215 nm. The stationary phase was a Kromasil KR 100-5C8 column (5 μm, 4.6 × 250 mm, EKA chemicals, Sweden) 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 were as follows: internal standard at 6.3 min, losartan at 11.4 min and EXP-3174 at 17.8 min. The calibration curves of losartan and EXP-3174 were linear within the range of 1–100 μg/ml. Recovery (%) assessed from the replicate analysis (n = 5) for five days by adding 20 μg/ml and 100 μg/ml of losartan to the rat plasma was shown 99.8 ± 4.8 and 98.1 ± 4.6, respectively. The lower limit of quantification for losartan and EXP-3174 in the rat plasma was 5 ng/ml. The coefficients of the variation for losartan and EXP-3174 were < 13.8% and < 14.3%, respectively.

2.4.2 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 [3]. Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrates 50 mM [7-Benzyloxy-4-(trifluoromethyl) couamrin (7-BFC) and 150 mM 7-Methoxy-4-trifluoromethyl coumarin (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.

2.4.3 Rhodamine-123 retention assay

MCF-7/ADR cells were seeded on 24-well plates at a seeding density of 10^5 cells. 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 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.

2.5. Pharmacokinetic analysis

The plasma concentration data were analyzed using a noncompartmental method on WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA), which uses the WinNonlin method to calculate the area under the curve (AUC) of the plasma concentration (C_p) as a function of time (t). The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were observed from the experimental data. The elimination rate constant (K_{el}) was estimated by regression analysis from the slope of the line of best fit, and the half-life ($t_{1/2}$) of the drug was obtained by 0.693/Kel. The area under the plasma concentration−time curve (AUC_{0-t}) from time zero to the time of the last measured concentration (C_{last}) was calculated using the linear trapezoidal rule. The AUC zero to infinity ($AUC_{0-\infty}$) was obtained by adding AUC_{0-t} and the extrapolated area was determined by C_{last}/K_{el}. The absolute bioavailability (A.B. %) of losartan was calculated by $AUC_{oral}/AUC_{iv} \times Dose_{i.v}/Dose_{oral} \times 100$, and the relative bioavailability (R.B. %) of losartan was estimated by $AUC_{losartan\ with\ silibinin} / AUC_{losartan} \times 100$. The metabolite-parent ratio (M.R.) was estimated by $(AUC_{EXP\text{-}3174} / AUC_{losartan}) \times (M.W._{losartan} / M.W._{EXP\text{-}3174})$.

2.6. Statistical analysis

All the means are presented with their standard deviations. The pharmacokinetic parameters were compared with one-way ANOVA, followed by a *posteriori* testing with the use of the Dunnett correction. A p value < 0.05 was considered statistically significant.

3. Results

3.1 Inhibition of CYP3A4 and CYP2C9

The inhibitory effect of silibinin on CYP3A4 and CYP2C9 activity is shown in Fig 2. Silibinin inhibited CYP3A4 and CYP2C9 enzymes activity in a concentration-dependent manner. Silibinin inhibited human CYP3A4 and CYP2C9 with IC50 values of 1.97 μM and 5.25 μM, respectively.

3.2 Rhodamine-123 retention assay

In this study, the cell-based P-gp activity test using rhodamine-123 also showed that silibinin (100 μM, $p < 0.01$) significantly inhibited P-gp activity (Fig. 3).

3.3 Effect of silibinin on the pharmacokinetics of losartan

The mean plasma concentration-time profiles of losartan in the presence or absence of silibinin are characterized in Fig.4. The mean pharmacokinetic parameters of losartan are also summarized in Table 1. As shown in Table 1, silibinin (1.5 or 6 mg/kg) significantly altered the pharmacokinetic parameters of losartan compared to those in the control group. Silibinin also significantly ($p < 0.05$, 1.5 mg/kg; $p < 0.01$, 6 mg/kg) increased the area under the plasma concentration–time curve (AUC0–∞) of losartan by 40.8–68.4% and the peak plasma concentration (Cmax) of losartan by 40.6–69.3%. Consequently, the absolute bioavailability (A.B.) of losartan in the presence of silibinin was 35.8–42.8%, which was enhanced significantly ($p < 0.05$) compared with that in the oral control group (25.4%). The relative bioavailability (R.B.) of losartan was increased by 1.41- to 1.68-fold over that in the control group. However, there was no significant change in the peak plasma concentration (Tmax) and the terminal half-life (t1/2) of losartan in the presence of silibinin.

3.4 Effect of silibinin on the pharmacokinetics of active metabolite, EXP-3174

As shown in Fig. 5 and summarized in Table 2, silibinin (6 mg/kg) significantly increased the AUC0–∞ (28.6%) of EXP-3174 compared to that in the control group. The metabolite-parent AUC ratio (M.R.) was significantly ($p < 0.05$) decreased by 32.1–35.8 % in the presence of silibinin (1.5 and 6 mg/kg) compared to that in the control group, implying that silibinin could effectively inhibit the CYP3A and CYP2C9-mediated metabolism of losartan.

4. Discussion

With the great interest in herbal products as alternative medicines, much effort is currently being expended to identify natural compounds of plant origin that modulate P-gp and metabolic enzymes. There is far less information on the pharmacokinetic interactions between herbal products and medicines. Based on the broad overlap in the substrate specificities as well as their co-localization in the small intestine, the primary site of absorption for orally administered drugs, CYP3A4 and P-glycoprotein have been recognized as a concerted barrier to drug absorption [4, 13]. Therefore, dual inhibitors against both CYP3A4 and P-gp may greatly impact on the bioavailability of many drugs for which CYP3A4 metabolism and P-gp-mediated efflux are the major barrier to systemic availability. In addition to its extensive metabolism by CYP3A4 and CYP2C9, losartan appeared to be a substrate of P-glycoprotein, suggesting that P-glycoprotein and CYP3A4 should act synergistically to limit the oral bioavailability of losartan [4, 27, 34]. Therefore, the present study evaluated the effect of silibinin, an antioxidant, on the bioavailability and pharmacokinetics of losartan in rats to examine a potential drug interaction between silibinin and losartan via the inhibition of CYP3A4 and CYP2C9 enzymes and P-gp [35].

The inhibition effect of silibinin against CYP3A4-mediated metabolism was confirmed by the employment of recombinant CYP3A4 enzyme. As shown in Fig. 2, silibinin exhibited inhibition effect against CYP3A4 and CYP2C9-mediated metabolism with IC50 of 1.97 and 5.25 μM, respectively. Furthermore, the cell-based assay using rhodamine-123 indicated that silibinin (100 μM) significantly ($p<0.01$) inhibited P-gp-mediated drug efflux (Fig. 3). These results are consistent with the previous report [6, 39]. Therefore, the pharmacokinetic characteristics of losartan were evaluated in the absence and the presence of silibinin in rats. As CYP3A9 expressed in rat is corresponding to the ortholog of CYP3A4 in human [16], rats' CYP3A2 are similar to human's CYP3A4 [1, 9]. Human CYP2C9 and 3A4 and rat CYP2C11 and 3A1 have 77 and 73%, respectively, protein homology

[18]. Rats were selected as an animal model in this study to evaluate the potential pharmacokinetic interactions mediated by CYP3A4, although there should be some extent of difference in enzyme activity between rat and human [2]. Therefore, silibinin might possible increase absorption of losartan in the intestine through the inhibition of P-gp and CYP3A4.

As shown in Table 1, silibinin (1.5 and 6 mg/kg) significantly enhanced the AUC of losartan. Since orally administered losartan is a substrate for CYP3A-mediated metabolism and P-gp-mediated efflux in the intestine and liver [30], silibinin might obstruct this metabolic pathway. Rajnarayana et al. [2, 16, 26] demonstrated that pretreatment with silymarin significantly changed the disposition of metronidazole and its active metabolite, hydroxymetronidazole. The pharmacokinetic profiles of EXP-3174 were also evaluated in the presence or absence of silibinin (Table 2). The metabolite-parent ratio (M.R.) in the presence of 1.5 and 6 mg/kg of silibinin decreased significantly ($p < 0.05$) compared to that in the control group, implying that the presence of silibinin could effectively inhibit the CYP3A or CYP2C9-mediated metabolism of losartan. These results are consistent with the report [29] that quercetin, an antioxidant, significantly decreased the M.R. of tamoxifen, a P-gp and CYP 3A substrate, in rats. The enhanced oral bioavailability of losartan in the presence of silibinin might be due to the inhibition of CYP3A and CYP2C9 enzymes and P-gp efflux transport by silibinin.

5. Conclusion

Silibinin enhanced the oral bioavailability of losartan in rats. Therefore, concomitant use of siliybinin with losartan will require close monitoring to potential drug interactions for the safe therapy. Clinical importance of these findings should be further investigated in clinical trials.

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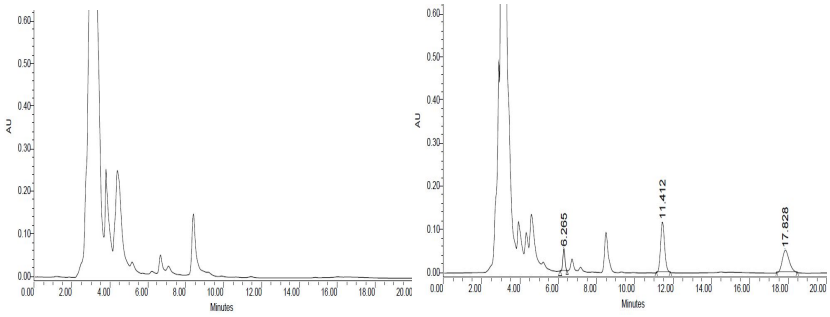

Figure 1. The HPLC chromatograms of the rat's blank plasma (A) and the p lasma spiked with L-158.809 (internal standard; 6.3 min), losartan (11.4 min) a nd EXP-3174 (17.8 min).

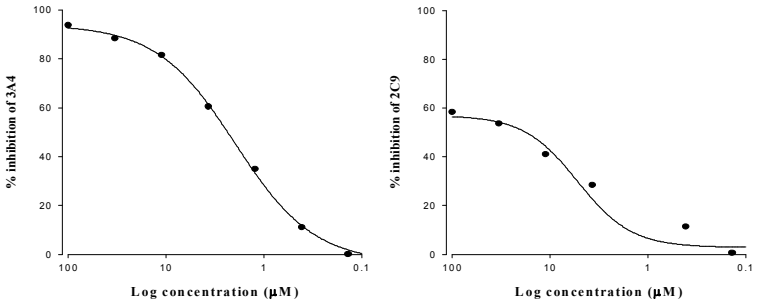

Figure 2. Inhibitory effect of silibinin on CYP3A4 (A) and 2C9 (B) activity. All experiments were performed in duplicate, and results are expressed as the percent of inhibition.

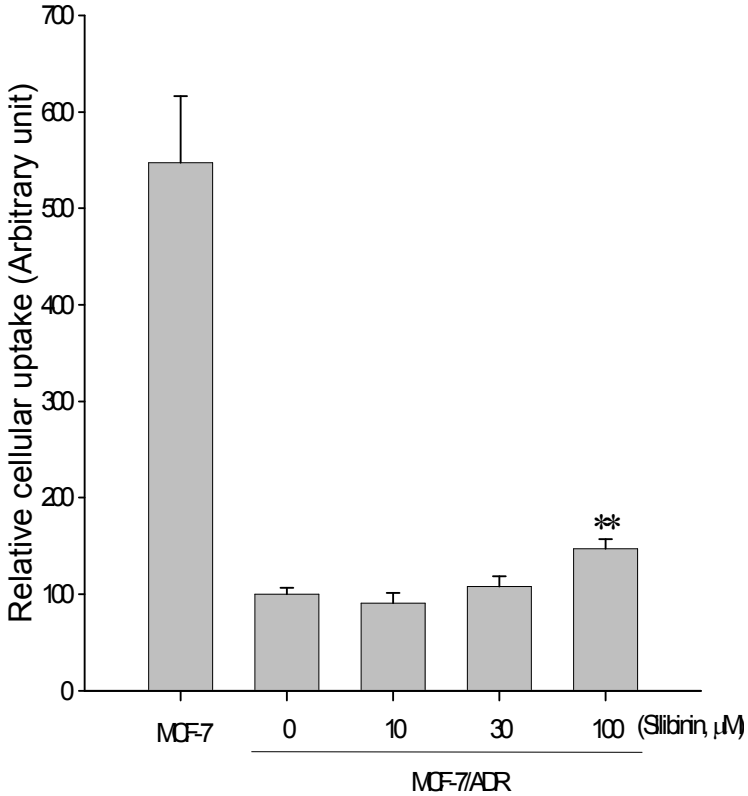

Figure 3. Effect of silibinin on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD of 6 separate samples (significant versus control MCF-7 cells, ** $p < 0.01$).

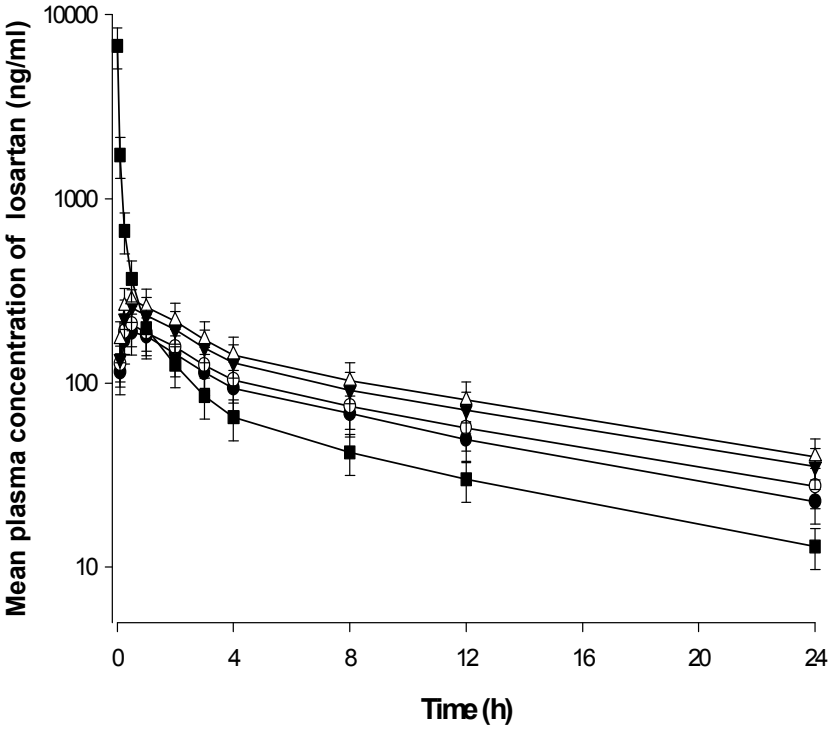

Figure 4. Mean plasma concentration-time profiles of losartan after intravenous (3 mg/kg) and oral (9 mg/kg) administration of losartan to rats in the presence or absence of r (0.3, 1.5 and 6 mg/kg) (mean ± SD, n = 6, each). Bars represent the standard deviation: (●) Oral administration of losartan (9 mg/kg; control); (○) with 0.3 mg/kg of silibinin; (▼) with 1.5 mg/kg of silibinin; (▽) with 6 mg/kg of silibinin; (■) Intravenous administration of losartan (3 mg/kg).

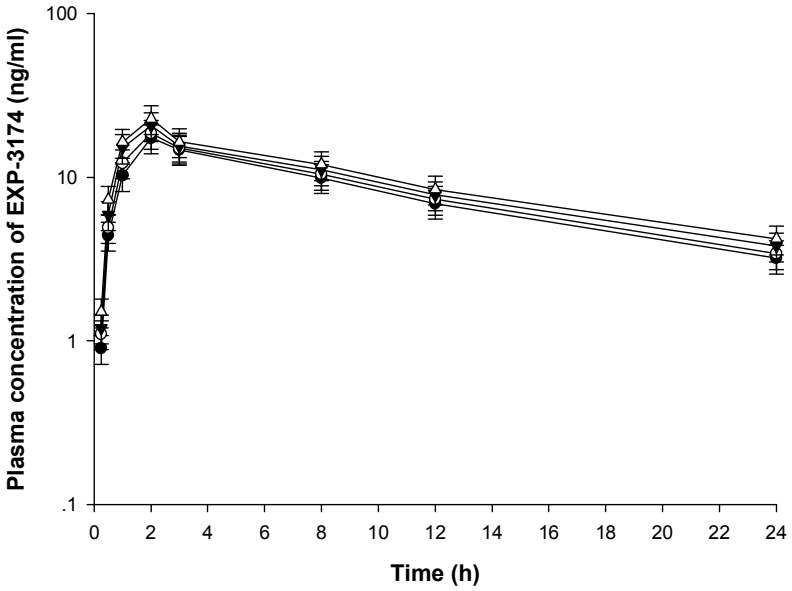

Figure 5. Mean plasma concentration-time profiles of EXP-3174 after oral (9 mg/kg) administration of losartan to rats in the presence or absence of silibinin (0.3, 1.5 and 6 mg/kg) (mean ± SD, n = 6, each). Bars represent the standard deviation: (●) Oral administration of losartan (9 mg/kg); (○) with 0.3 mg/kg of silibinin; (▼) with 1.5 mg/kg of silibinin; (△) with 6 mg/kg of silibinin.

Table 1. Mean pharmacokinetic parameters of losartan after intravenous (3 mg/kg) or oral (9 mg/kg) administration of losartan to rats in the presence or absence of silibinin (mean ± SD, *n* = 6)

Parameters	Losartan (Control)	Losartan + Silibinin			Losartan (i.v.)
		0.3 mg/kg	1.5 mg/kg	6 mg/kg	
$AUC_{0-\infty}$ (ng ·h/ml)	228 ± 43	262 ± 48	321 ± 63*	384 ± 72**	299 ± 61
C_{max} (ng/ml)	19.2 ± 4.2	22.3 ± 4.4	27.0 ± 5.2*	32.5 ± 5.8**	ND
$t_{1/2}$ (h)	10.7 ± 2.6	10.9 ± 3.1	11.0 ± 3.3	11.2 ± 3.6	8.7 ± 1.9
T_{max} (h)	1.08 ± 0.25	1.08 ± 0.25	1.00 ± 0.55	0.83 ± 0.26	ND
A.B. (%)	25.4 ± 5.4	29.2 ± 5.9	35.8 ± 7.4*	42.8 ± 8.6**	100
R.B. (%)	100	115	141	168	ND

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

AB,absolute bioavailability ; $AUC_{0-\infty}$, area under the plasma concentration-time curve from o h to infinity; C_{max} , peak plasma concentration; RB, relative bioavailability; SD, standard deviation; T_{max} time to reach peak plasma concentration; $t_{1/2}$ terminal half life.

Table 2. Mean pharmacokinetic parameters of EXP-3174 after oral administration of losartan (9 mg/kg) to rats in the presence and absence of silibinin (mean ± SD, *n* = 6)

Parameters	Losartan (Control)	Losartan + Silibinin		
		0.3 mg/kg	1.5 mg/kg	6 mg/kg
$AUC_{0-\infty}$ (ng ·h/ml)	230 ± 51	244 ± 58	269 ± 62	296 ± 66*
C_{max} (ng/ml)	17.3 ± 4.1	18.5 ± 4.6	20.6 ± 5.0	22.7 ± 5.3
T_{max} (h)	2.33 ± 0.52	2.17 ± 0.76	2.00 ± 0.63	2.00 ± 0.63
$t_{1/2}$ (h)	9.6 ± 2.4	9.8 ± 2.6	10.4 ± 3.0	10.6 ± 3.1
M.R.	1.06 ± 0.25	0.92 ± 0.23	0.72 ± 0.18*	0.68 ± 0.16*

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

$AUC_{0-\infty}$, area under the plasma concentration-time curve from o h to infinity; C_{max}, peak plasma concentration; RB, relative bioavailability; SD, standard deviation; T_{max} time to reach peak plasma concentration; $t_{1/2}$ terminal half life; M.R., metabolite-parent ratio.

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저작물 이용 허락서

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논문제목	한글: 흰쥐에서 항산화제인 실리비닌이 로살탄의 생체이용률에 미치는 영향 영문: Effects of silibinin, an antioxidant, on the bioavaility of Losartan 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 8 월

저작자: 이재성 (서명 또는 인)

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