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EFFECTS OF APIGENIN ON THE BIOAVAILABILITY OF LOSARTAN IN RATS

조선대학교 대학원

약학과

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흰쥐에서 아피제닌이 로살탄의 생체이용율에 미친 영향

2009 년 2 월 25 일

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이 논문을 약학석사학위신청 논문으로 제출함.

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Abstract

The present study was to investigate the effect of apigenin, a flavonoid, on the pharmacokinetics of losartan and its active metabolite, EXP-3174, in rats. Pharmacokinetic parameters of losartan and EXP-3174 in rats were determined after an oral administration of losartan (1 mg/kg) in the presence or absence of apigenin (0.5, 2.5 and 10 mg/kg). The pharmacokinetic parameters of losartan were significantly altered by the presence of apigenin compared with the control group (given losartan alone). Presence of apigenin significantly ($p < 0.05$, 2.5 mg/kg; $p < 0.01$, 10 mg/kg) increased the area under the plasma concentration–time curve (AUC) of losartan by 19.9–91.6% and peak plasma concentration (C_{max}) of losartan by 14.8–78.8%. Consequently, the absolute bioavailability (AB) of losartan in the presence of apigenin was 38.9–63.2%, which was enhanced significantly ($p < 0.05$) compared with the oral control group (32.9%). The relative bioavailability (R.B.) of

losartan increased by 1.26- to 1.92-fold in the presence of apigenin. However, there was no significant change in the peak plasma concentration (T_{max}) and terminal half-life ($t_{1/2}$) of losartan in the presence of apigenin. Presence of apigenin (10 mg/kg) significantly increased the AUC (41.1%) of EXP-3174 compared with the control group. Metabolite-parent AUC ratio in the presence of apigenin (10 mg/kg) significantly ($p < 0.05$) decreased by 26.3 % compared to the control group, implying that presence of apigenin could be effective to inhibit the cytochrome P450 (CYP)3A4-mediated metabolism and P-glycoprotein (P-gp)- mediated efflux of losartan. In conclusion, the presence of apigenin significantly enhanced the oral exposure of losartan, suggesting that concurrent use of apigenin or apigenin-containing dietary supplement with losartan should require close monitoring for potential drug interactions.

Key words**:** Losartan, EXP-3174, Apigenin, Pharmacokinetics, CYP3A4, P-gp, Rat

국 문 초 록

흰쥐에서 아피제닌이 로살탄의 생체이용율에 미친 영향

임 진 영

지 도 교 수: 최 준 식

조선대학교대학원 약학과

본 연구에서는 항산화제, 항염증제인 아피제닌이 고혈압치료제인 로살탄의 약물동태에 미치는 영향을 연구검토하였다. 아피제닌(0.5, 2.5 및 10 mg/kg)과 로살탄을 흰쥐에 경구(1 mg/kg) 및 정맥(0.3 mg/kg)으로 병용투여하여 본 연구를 실시하였다. 혈장농도곡선하면적(AUC) 및 최고혈중농도(C_{max})는 대조군에 비해 아피제닌 병용투여군에서 유의성($P<0.05$) 있게 증가하였다. 대조군에 비해 아피제닌 병용투여군에서 절대적생체이용율(AB)은 유의성($P<0.05$) 있게 증가되었다. 그 결과로 상대적생체이용율(RB)이 1.26-1.92 배 증가되었다. 이 결과는 아피제닌이 소장 또는 간장에서 CYP3A4와 P-gp를 억제시킴으서 로살탄의 생체이용율이 증가된것으로 사료된다. 아피제닌의 용량증가와 더불어 로살탄의 생체이용율도 증가되었다. 임상에서 아피제닌과 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 faeces 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].

Flavonoids represent a group of phytochemicals that are produced by various plants in high quantities [18]. Among flavonoids, apigenin (4′,5,7-trihydroxyflavone) exhibits various biological activities including antioxidation, anti-mutagenesis and anti-inflammation [19-21]. Furthermore, apigenin was reported to modulate metabolic enzymes (CYP3A4) as well as P-gp efflux pump [22-26]. Nguyen et al. also reported that apigenin significantly increased the cellular accumulation of vinblastine, a P-gp substrate in Panc-1 cells [27].

Therefore, apigenin is effective dual inhibitor of CYP3A4 and P-gp.

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

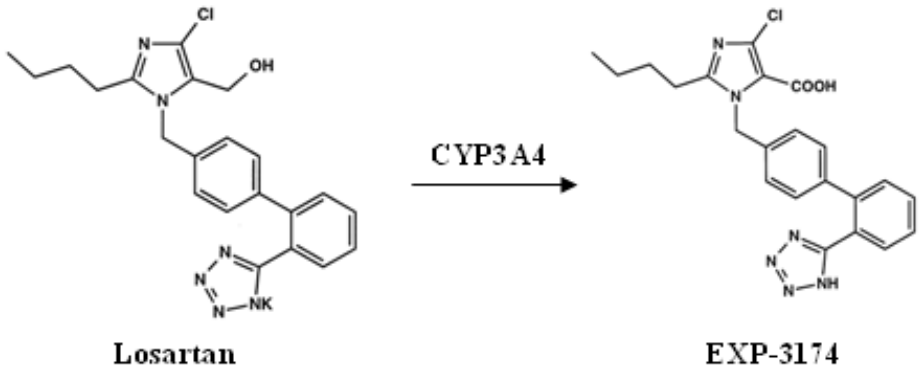

Figure 1. The metabolic pathway of losartan to EXP-3174 in rats.

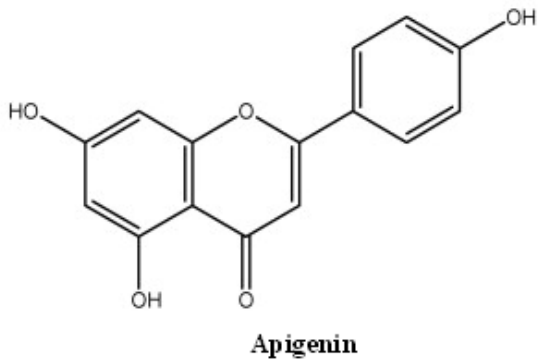

Figure 2. Chemical structure of apigenin.

2. Materials and methods

2.1. Chemicals

Losartan, EXP-3174 and L-158.809 (internal standard) were obtained from the Merck Co. (Darmstadt, Germany). Apigenin 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^{o}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 1 mg/kg without or with oral administration of apigenin at a dose of 0.5, 2.5 or 10 mg/kg. 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 apigenin 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), 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 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. HPLC analysis of losartan and EXP-3174

2.3.1. Sample preparation

Plasma concentrations of losartan were determined using a slightly modified of the reported HPLC assay reported by Zarghi et al [28]. Briefly, a 50 μl aliquot of L-158.809 (0.2 μg/ml; internal standard), and a 0.5 ml aliquot of acetonitrile were added to a 0.2 ml aliquot of samples. The mixture was then stirred for 2 min and centrifuged at 13,000 rpm, for 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. 50 μl aliquot of the water layer was injected into the HPLC system.

2.3.2. HPLC condition

The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu, Japan), a UV-Vis detector (Model SPD-10A), a system controller (Model SCL-10A), a degasser (Model DGU-12A) and an autoinjector (SIL-10AD). The mobile phase was acetonitrile : 0.01 M phosphate buffer (70 : 30, v/v, pH 3, adjusted with phosphoric acid) and was run to flow rate of 2 ml/min. The column was Kromasil KR 100-5C_8 column (5 μm, 4.6 × 250 mm, EKA Chemicals, Sweden) and UV detector was set at 254 nm at room temperature. The retention

times at a flow rate of 2 ml/min are as follows: internal standard at 5.3 min, losartan at 11.0 min and EXP-3174 at 21.0 min (Figure 3). The lower limit of quantification for losartan and EXP-3174 in the rat plasma was 10 ng/ml. The coefficients of the variation of losartan and EXP-3174 were less than 13.9% and 15.9%, respectively (Figure 4 and Figure 5).

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 viariance (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.

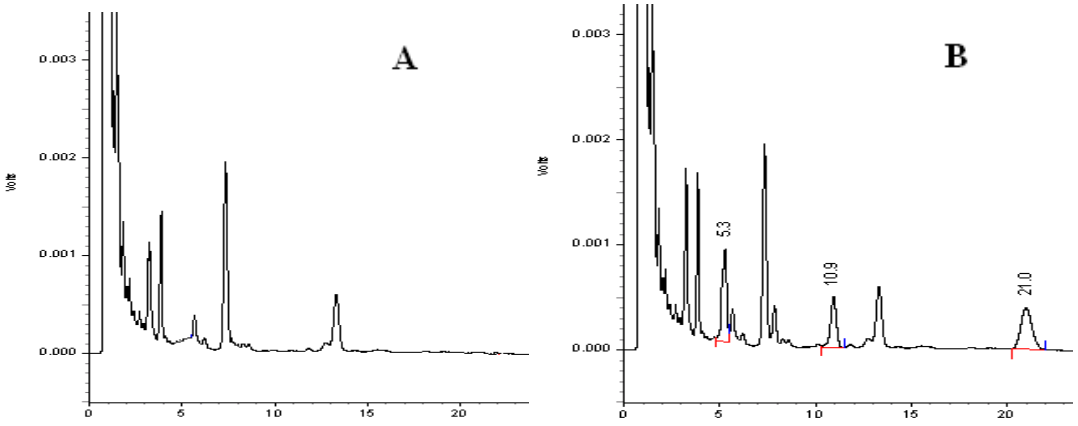

Figure 3. HPLC chromatograms of the rat's blank plasma (A), and the plasma spiked with losartan (10.9 min), EXP-3174 (21.0 min), and L-158.809 (internal standard; 5.3 min) (B).

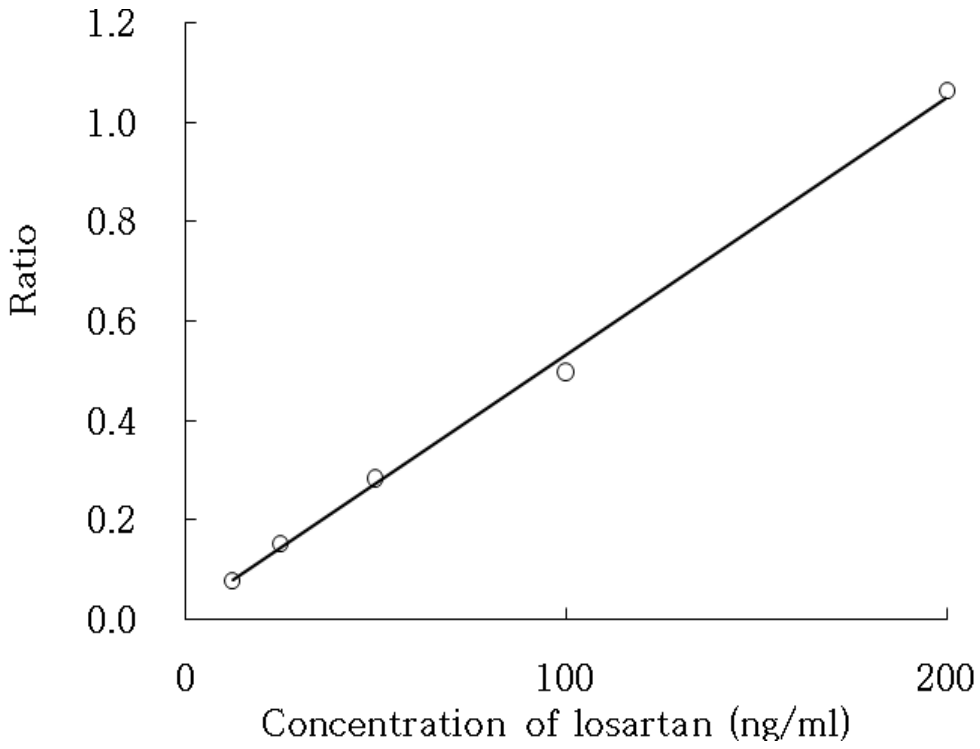

Figure 4. A calibration curve of losartan when spiked into the rat's blank plasma.

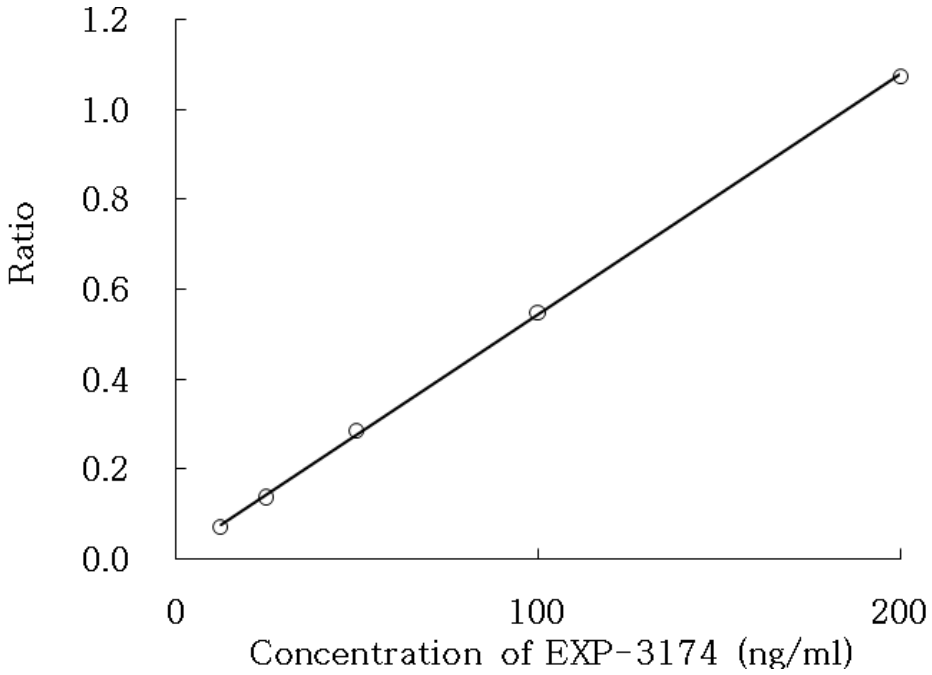

Figure 5. A calibration curve of EXP-3174 when spiked into the rat's blank plasma.

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

Time (h)	Control (Losartan)	Losartan + Apigenin		
		0.5 mg/kg	2.5 mg/kg	10 mg/kg
0	0	0	0	0
0.1	115 ± 28.8	133 ± 34.6	181 ± 47.1	205 ± 53.3
0.25	169 ± 43.9	198 ± 51.5	272 ± 70.7	311 ± 77.8
0.5	189 ± 47.2	217 ± 56.4	298 ± 77.5	338 ± 87.9
1	180 ± 46.8	196 ± 20.5	270 ± 70.2	307 ± 79.8
2	144 ± 36	164 ± 42.6	226 ± 58.8	257 ± 66.8
3	114 ± 29.6	130 ± 32.5	180 ± 45	205 ± 53.3
4	94 ± 24.4	108 ± 28.1	149 ± 38.7	169 ± 42.3
8	71 ± 18.5	81 ± 21.1	111 ± 28.9	127 ± 33
12	52 ± 13	63 ± 15.8	87 ± 22.6	99 ± 24.8
24	24 ± 6.2	30 ± 7.8	43 ± 11.2	48 ±12.5

Table 2. Mean (± S.D.) plasma concentrations of EXP-3174 after oral administration of losartan (1 mg/kg) with or without of apigenin to rats (n = 6, each).

Time (h)	Control (EXP-3174)	Losartan + Apigenin		
		0.5 mg/kg	2.5 mg/kg	10 mg/kg
0	0	0	0	0
0.1	16.8 ± 4.2	18.5 ± 4.8	20.4 ± 5.3	23.5±6.1
0.25	21.2 ± 5.5	23 ± 5.9	25.0 ± 6.25	30.6±7.9
0.5	34.6 ± 8.9	37.1 ± 9.6	39.9 ± 10.4	45.9±11.5
1	44.4 ± 11.1	47.8 ± 11.9	52.0 ± 13.5	59.0±15.3
2	42.0 ± 10.9	44.3 ± 11.5	47.0 ± 12.2	53.9±13.5
3	37.6 ± 9.8	39.4 ± 10.2	42.0 ± 10.9	47.1±12.2
4	31.4 ± 7.9	33.3 ± 8.7	36.0 ± 9.4	42.0±10.5
8	26.5 ± 6.9	28.4 ± 7.4	30.8 ± 8.0	35.0±9.1
12	19.8 ± 5.1	21.3 ± 5.3	23.7 ± 5.9	27.6±6.9
24	9.7 ± 2.5	10.7 ± 2.8	12.1 ± 3.0	14.2±3.7

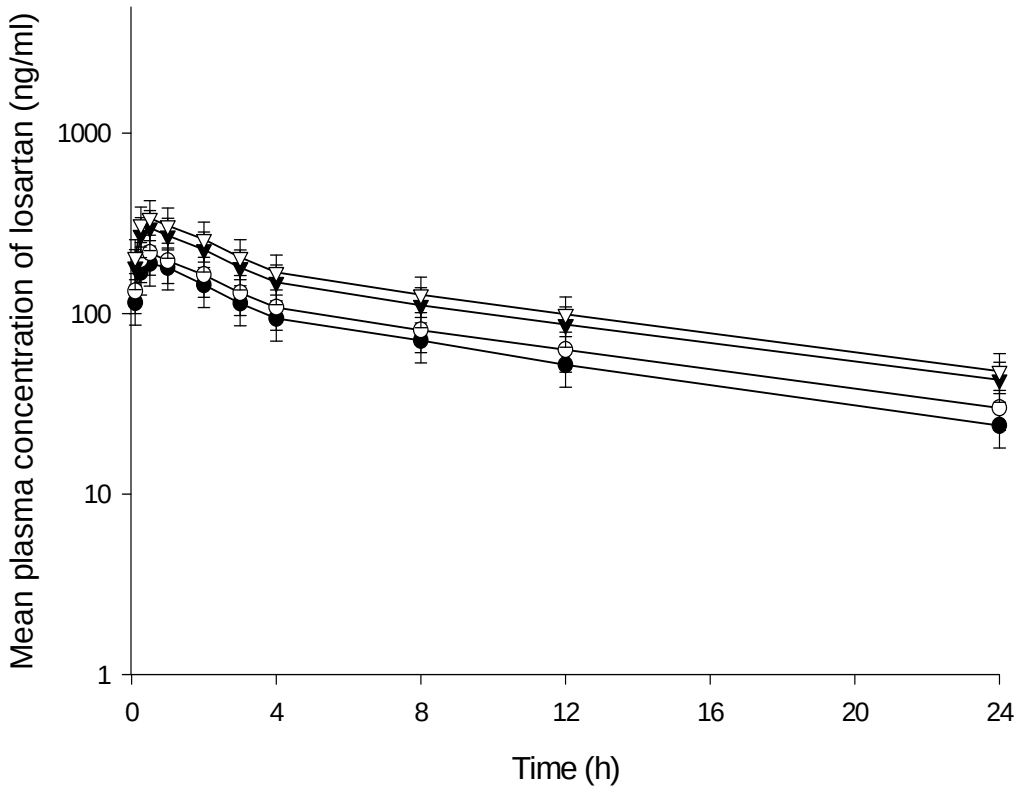

Figure 6. Mean arterial plasma concentration–time profiles of losartan after oral administration of losartan (1 mg/kg) without (●) or with 0.5 mg/kg (○) or 2.5 mg/kg (▼) or 10 mg/kg (▽) of apigenin to rats (*n* = 6, each). Bars represent the standard deviation.

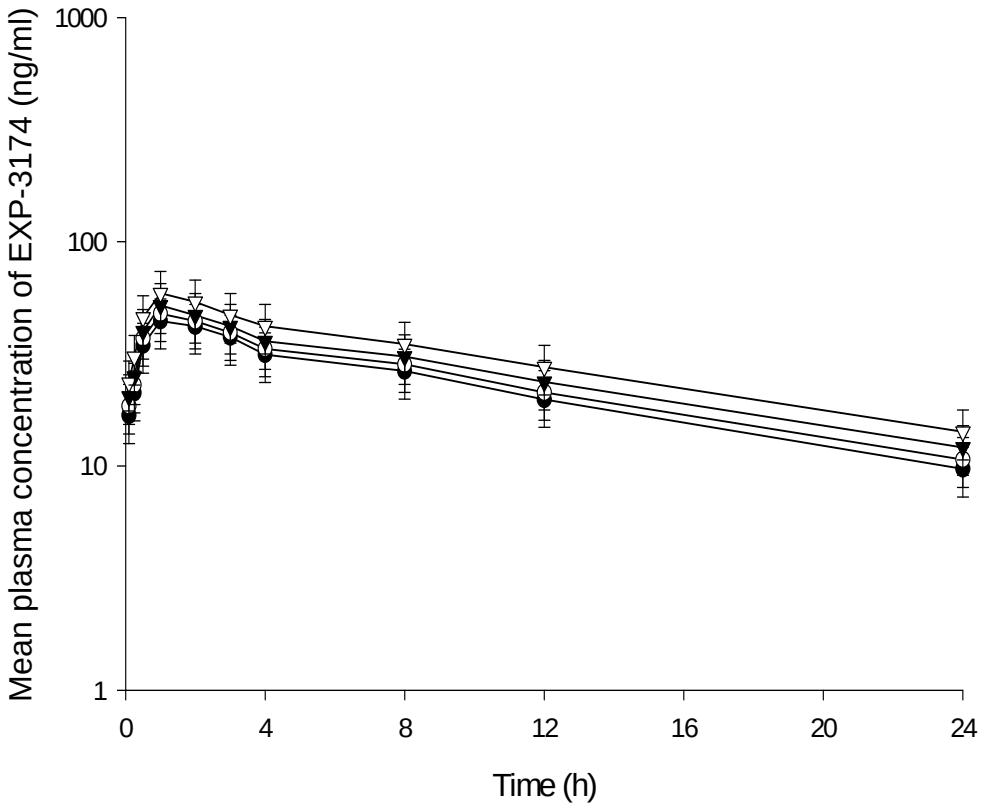

Figure 7. Mean arterial plasma concentration–time profiles of EXP-3174 after oral administration of losartan (1 mg/kg) without (●) or with 0.5 mg/kg (○) or 2.5 mg/kg (▼) or 10 mg/kg (▽) of apigenin to rats (*n* = 6, each). Bars represent the standard deviation.

Table 3. Mean (± S.D.) pharmacokinetic parameters of losartan after oral administration of losartan (1 mg/kg) with or without of apigenin to rats (*n* = 6, each).

Parameter	Control	Losartan + Apigenin		
		0.5 mg/kg	2.5 mg/kg	10 mg/kg
AUC (ng·h/ml)	1943 ± 505	2329 ± 605	3269 ± 849*	3723 ± 967**
C_{max} (ng/ml)	189 ± 47.3	217 ± 56.4	298 ± 77.5**	338 ± 87.9**
T_{max} (h)	0.5	0.5	0.5	0.5
$t_{1/2}$ (h)	10.2 ± 2.7	10.9 ± 2.8	11.3 ± 2.9	11.4 ± 2.9
RB (%)	100	126	168	192
AB (%)	32.9	38.9	55.4**	63.2**

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

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

C_{max}: peak plasma concentration;

T_{max}: time to reach C_{max};

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

RB: relative bioavailability.

AB : absolute bioavailbility

Table 4. Mean (± S.D.) Pharmacokinetic parameters of EXP-3174 after oral administration of losartan (1 mg/kg) with or without of apigenin to rats (*n* = 6, each).

Parameter	Control	Losartan + Apigenin		
		0.5 mg/kg	2.5 mg/kg	10 mg/kg
AUC (ng·h/ml)	695 ± 125	756 ± 144	843 ± 177	981 ± 215*
C_{max} (ng/ml)	44.4 ± 7.9	47.8 ± 9.1	52 ± 10.9	59 ± 13.0*
T_{max} (h)	1	1	1	1
$t_{1/2}$ (h)	11.3 ± 2.0	11.9 ± 2.3	12.4 ± 2.6	12.6 ± 2.8
MR(%)	35.8 ± 6.5	32.5 ± 6.3	25.8 ± 5.8	26.4 ± 5.4*

* $P < 0.05$ compared to control.

$AUC_{0-\infty}$: area under the plasma concentration–time curve from 0 h to infinity;

C_{max}: peak plasma concentration;

T_{max}: time to reach C_{max};

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

MR: metabolite-parent ratio.

3. Results and Discussion

The calibration curves of losartan (Figure 4) and EXP-3174 (Figure 5) were linear within the concentration ranges from 10–200 ng/ml, respectively. The detection limits for losartan and EXP-3174 was 10 ng/ml. The coefficients of the variation of losartan and EXP-3174 were less than 13.9% and 15.9%, respectively (Figure 4 and Figure 5).

Figure 6 shows the mean plasma concentration–time profiles of losartan after oral administration (1 mg/kg) with or without of apigenin (0.5, 2.5 and 10 mg/kg), and Table 3 lists the relevant pharmacokinetic parameters of losartan after oral administration. Apigenin significantly (2.5 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased the area under the plasma concentration–time curve (AUC) of losartan. Apigenin significantly ($P < 0.05$) increased the peak concentration (C_{max}) of losartan. Consequently, the relative bioavailability (RB) of losartan increased by 1.26– to 1.92–fold in the presence of apigenin. CYP3A4, a key enzyme 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 CYP2C8 to several active and inactive metabolites [7]. *In vitro*, apigenin has been reported to inhibit CYP3A4 enzymes [22, 23]. The enhanced bioavailability of losartan by apigenin might be due to the competitive inhibition of CYP3A4. This result appeared to be consistent with previous studies reported by Piao *et al*. and Choi *et al*. [30, 31]; a single oral administration of morin and naringin significantly increased the AUC and C_{max} of nicardipine and diltiazem in rats, respectively. Which was due to inhibition of CYP3A4 and P-gp in the intestine and/or liver. However, there was no significant change in the peak plasma concentration (T_{max}) and terminal half-life ($t_{1/2}$) of losartan in the presence of apigenin.

Figure 7 depicts the mean plasma concentration–time profiles of EXP-3174 after

oral administration of losartan (1 mg/kg) with or without apigenin (0.5, 2.5 and 10 mg/kg). As listed in Table 4, presence of apigenin (10 mg/kg) significantly increased the AUC (41.1%) of EXP-3174 compared with the control group. Metabolite-parent AUC ratio in the presence of apigenin (10 mg/kg) significantly ($p < 0.05$) decreased by 26.3 % compared to the control group, implying that coadministration of apigenin could be effective to inhibit the cytochrome P450 (CYP)3A4-mediated metabolism and P-glycoprotein (P-gp) mediated efflux of losartan. Presence of apigenin did not change the T_{max} 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 [31].

The increased bioavailability of losartan by apigenin suggests that CYP3A or P-gp could be competitively inhibited by apigenin, which resulted in reducing first-pass extraction of losartan in the intestine and/or liver. The adjustment of the dose of losartan should be taken into consideration for potential interaction between apigenin and losartan in clinical setting.

4. Conclusion

Presence of apigenin significantly enhanced the systemic exposure of losartan in rats. If the results are further confirmed in the clinical trial, dose adjustment of losartan shoud be taken into consideration when losartan is treated with concomitantly with apigenin to the patients.

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힘든 대학원 생활동안 저의 힘이 되어준 선배 박영길, 서기수 그리고 동기 임영빈, 고운정에게도 깊은 감사의 말씀 드립니다. 대학원 재학과 실험으로 인해 자주 만나지 못해 항상 아쉬운 나의 친구 현욱, 재하, 대웅, 선의 그리고 어려울 때마다 고민상담을 해준 선배이자 친구 기수, 연구를 하면서 난관에 봉착했을때 고민을 풀어주신 박영길 선생님, 모두모두 감사의 말씀 전하고 싶습니다.

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저작물 이용 허락서

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논문제목	한글: 흰쥐에서 아피제닌이 로살탄의 생체이용율에 미친영향 영문: Effects of apigenin on the bioavailability 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 ( )

2009 년 2 월

저작자: 임진영 (서명 또는 인)

조선대학교 총장 귀하