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국 문 초 록

흰쥐에서 캠페롤과 리코찰콘 A가 니페디핀의 생체이용효율에 미치는 영향

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채소 및 과일류에 함유되어 있는 항산화제인 캠페롤 또는 약용식물 중 빈용되는 감초성분인 리코찰콘 A와 고혈압 약물인 니페디핀과 병용되는 경우가 있으므로 이에 대한 상호작용을 알아보고자, 흰쥐에 니페디핀 (경구; 10 mg/kg, 정맥; 2.5 mg/kg)과 캠페롤 및 리코찰콘 A (0.4, 2, 10 mg/kg)을 병용 경구 투여한 후 니페디핀 및 그 주대사체인 디히드로니페디핀의 생체이용효율 및 약물동태학적 변수들을 대조군과 비교 검토하였다.

캠페롤과 리코찰콘 A는 cytochrome P450 (CYP) 3A4 효소억제작용과 P-glycoprotein (P-gp) 기능억제작용이 있었으며. 리코찰콘 A는 캠페롤 보다 CYP3A4 억제작용과 P-gp억제작용이 더 강했다.

캠페롤 또는 리코찰콘 A와 병용투여 시 니페디핀의 생체이용효율 및 약물동태학적 변수는 유의성 있게 변화하였다. 대조군에 비해 캠페롤 또는 리코찰콘 A (2, 10 mg/kg)와 병용투여군에서 니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 각각 유의성 있게 증가되었다. 캠페롤 또는 리코찰콘 A와 병용 투여 시 니페디핀의 전신클리어런스 (CL/F)는 유의성 있게 각각 감소되었다.

절대적 생체이용률 (AB)도 대조군에 비해 각각 유의성 있게 증가되었다. 캠페롤 또는 리코찰콘 A (10 mg/kg)와 니페디핀을 병용투여한군에서 대조군에 비해 주 대사체인 디히드로니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)이 유의성 있게 증가되었다. 그리고 캠페롤 또는 리코찰콘 A는 니페디핀의 대사율 (MR)을 유의성 있게 감소시켰다.

정맥투여군에서는 캠페롤 또는 리코찰콘 A와 병용투여가 니페디핀의 생체이용효율 및 약물동태학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 항산화 작용이 있으며 폴리페놀류인 캠페롤 또는 리코찰콘 A를 고혈압치료제인 니페디핀과 병용투여 하였을 때 경구 투여된 니페디핀의 생체이용효율이 유의성 있게 증가되었으며, 그 이유는 캠페롤 또는 리코찰콘A에 의해서 주로 소장에 존재하는 P-gp억제에 의한 흡수증가와 주로 소장과 간장에 존재하는 CYP3A4 억제에 의한 니페디핀의 초회통과효과 (대사)감소 또는 전신클리어런스 감소에 기인한 것으로 사료된다.

그러므로, 니페디핀과 캠페롤 또는 리코찰콘 A를 병용경구투여 하거나 혹은 캠페롤 또는 리코찰콘 A가 함유된 식품을 함께 병용경구투여 할 때 니페디핀의 약물동태에 미치는 영향을 고려하여 용량을 조절하는 것이 바람직하다고 사료된다.

Introduction

Nifedipine (dimethyl2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a calcium channel-blocking agent that is widely used for the treatment of essential hypertension, coronary artery spasm, and angina pectoris[1]. It inhibits the influx of extracellular calcium through myocardial and vascular membrane pores by physically plugging the channel, resulting in decreased intracellular calcium levels, inhibition of the contractile processes of smooth muscle cells, dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, and decreased total peripheral resistance, systemic blood pressure, and afterload [2, 3].

In humans, nifedipine is predominantly metabolized by cytochrome P450 (CYP) 3A4 to its primary pyridine metabolite, dehydronifedipine[4, 5]. CYP enzymes are responsible for the oxidative metabolism of many xenobiotics and play a major role in the phase I metabolism of many drugs[6]. CYP3A4 is the most abundant CYP enzyme (30-40%) in adult liver and metabolizes more than 50% of the clinically used drugs including nifedipine, cyclosporine, midazolam, and erythromycin[7, 8]. There are some reports that nifedipine is a substrate of CYP3A4 in human[9-11]. P-glycoprotein (P-gp) is an adenosine-50-triphosphate (ATP) dependent efflux drug transporter that is constitutively expressed in normal tissues that includes gastrointestinal epithelium, canalicular membrane of the liver, kidney[12, 13] and capillary endothelial cells in the central nervous system [14, 15]. Because of such tissue localized and its broad substrate specificity, P-gp appears to play a key role in absorption, distribution, and elimination of many drugs[16, 17]. It is generally known that the substrate and/or inhibitors of CYP3A4 and P-gp overlap with each other[18]. Dorababu et al.[19] reported that nifedipine belonged to a group of P-gp substrate. Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically to promote presystemic drug metabolism, resulting in the limited absorption of drugs.

Flavonoids represent a group of phytochemicals that are produced in high quantities by

various plants[20]. These compounds exhibit a wide range of beneficial biological activities including antioxidative, radical scavenging, antiatherosclerotic, antitumor and antiviral effects [21]. Flavonoids also modulate the CYP 3A subfamily and/or P-gp[22-24].

Kaempferol (3,4',5,7-tetrahydroxyflavone) is a natural flavonol, a type of flavonoid (Figure 1)[25]. Kaempferol is most widely distributed in onion, pumpkin, carrot, and black tea; contents of kaempferol were 832, 370,140, and 118 mg/kg of dry weight, respectively[25]. Kaempferol is also a CYP3A4 inhibitor[26]. Kaempferol has been reported to be a substrate[27] and an inhibitor[28] of P-gp. But the effect of kaempferol on CYP3A4 and P-gp inhibition is partially ambiguous. Thus, we reevaluated CYP3A4 and P-gp activity using rhodamine-123 retention assay in P-gp overexpressed adriamycin-resistant human breast cancer cell line (MCF-7/ADR).

For example, kaempferol exhibited a remarkable inhibition of P-gp-mediated efflux of ritonavir, thus cellular uptake of ritonavir increased[29]. Licochalcone A is a chalconoid, a type of natural phenols (Figure 1) [30]. Licochalcone A is an estrogenic flavonoid and the main active compound of the licorice species *Glycyrrhiza inflate*[31]. Licochalcone A also has a wide range of biological and pharmacological activities, including antioxidant, superoxide scavenging[30], anti-leishmanial activity, and effects on the function of parasite mitochondria[32], antimalarial activities both *in vitro* and *in vivo*[33], and antitumor activities in cancer cells[34-36]. Kwon *et al*. demonstrated that licochalcone A exerts anti-inflammatory effects by suppressing nuclear factor-kB (NF-kB) and activator protein-1 (AP-1) signaling[37]. Kim *et al*. provide the first evidence that licochalcone A could inhibit the angiogenesis *in vitro* and *in vivo* and the tumor growth[38]. Thus, it could be expected that kaempferol and licochalcone A would change bioavailability and pharmacokinetics of nifedipine by inhibiting P-gp and CYP3A4 in rats.

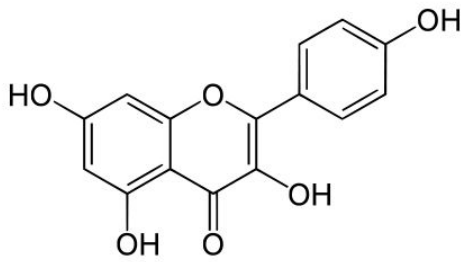
Furthermore, since kaempferol and licochalcone A have become readily available over-the-counter, it is possible that kaempferol and licochalcone A products would be taken along with prescribed nifedipine for the therapy of cardiovascular disease. Therefore, the aim of this study was to examine the effects of kaempferol and licochalcone A on the CYP3A4, P-gp activity and bioavailability and pharmacokinetics of nifedipine after oral and intravenous administration with kaempferol and licochalcone A in rats.

We evaluated CYP enzymes activities and P-gp activity about kaempferol and licochalcone A using CYP inhibition assays and rhodamine-123 retention assays in P-gp-over-expressed MCF-7/ADR cells. Kaempferol and licochalcone A are expected to change the bioavailability and pharmacokinetics of drugs those are substrates of P-gp and/or CYP3A4, if they are used concomitantly.

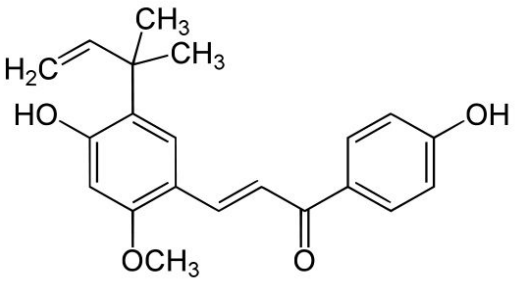
Nifedipine and kaempferol, licochalcone A interact with CYP enzymes and P-gp, and the increased use of health supplements may result in kaempferol and licochalcone A being taken concomitantly with nifedipine to treat or prevent cardiovascular diseases as a combination therapy. It is important to assess the potential bioavailability and pharmacokinetics interactions after the concurrent use of nifedipine and kaempferol, licochalcone A or kaempferol and licochalcone A containing dietary supplement in order to assure the effectiveness and safety of drug therapy. Antihypertensive agents are commonly co-administered with kaempferol and licochalcone A, herbar medicine, for the prevention or treatment of cardiovascular diseases as a combination therapy in clinics. But there are fewer reports about the effects of herbar medicine, over-the-counter (OTC) medicine, on the bioavailability or pharmacokinetics of antihypertensive drugs in rats. For example, effect of grapefruit juice on the pharmacokinetics of losartan and its active metabolite E3174 in healthy volunteers[39], effect of epigallocatechin gallate on the oral pharmacokinetics of verapamil in rats[40], enhanced bioavailability of verapamil after oral administration with hesperidin in rats[41], pharmacokinetic interaction between diltiazem and morin, a flavonoid, in rats[42], Effect of hesperidin on the oral pharmacokinetics of diltiazem and its main metabolite[43], desacetyldiltiazem, in rats, Effects of myricetin, an antioxidant, on the pharmacokinetics of losartan and its active metabolite, EXP-3174, in rats[44] and Effects of morin on the pharmacokinetics of nicardipine after oral and intravenous administration of nicardipine in rats[45].

However, the effect of kaempferol and licochalcone A on the bioavailability and pharmacokinetics of nifedipine *in vivo* has not yet been reported. Thus, the purpose of this study is to investigate the possible effects of kaempferol and licochalcone A on the CYP3A4 and P-gp activity and bioavailability or the pharmacokinetics of nifedipine and its active

metabolite, dehydronifedipine, after oral and intravenous administration of nifedipine with kaempferol and licochalcone A in rats.



(A) kaempferol



(B) licochalcone A

Figure 1. Structure of kaempferol (A) and licochalcone A (B).

Part I. Effects of kaempferol on the bioavailability of nifedipine in rats

국 문 초 록

채소 및 과일류에 함유되어 있는 항산화제인 캠페롤과 고혈압 약물인 니페디핀이 병용되는 경우가 있으므로 이에 대한 상호작용을 알아보고자, 흰쥐에 니페디핀 (경구; 10 mg/kg, 정맥; 2.5 mg/kg)과 캠페롤 (0.4, 2, 10 mg/kg)을 병용경구투여한 후 니페디핀 및 그 주대사체인 디히드로니페디핀의 생체이용효율 및 약물동태학적 변수들을 대조군과 비교 검토하였다.

캠페롤과 병용 투여 시 니페디핀의 생체이용효율 및 약물동태학적 변수는 유의성 있게 변화하였다. 대조군에 비해 캠페롤 (2, 10 mg/kg)과 병용투여군 에서 니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 각각 유의성 있게 증가되었으며 전신클리어런스 (CL/F)는 유의성 있게 감소되었다.

절대적 생체이용률 (AB)도 대조군에 비해 각각 유의성 있게 증가되었다. 캠페롤 (10 mg/kg)과 니페디핀을 병용투여한군에서 대조군에 비해 주대사체인 디히드로니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)이 유의성 있게 증가되었다. 그리고 캠페롤은 니페디핀의 대사율 (MR)을 유의성 있게 감소시켰다.

정맥투여군에서는 캠페롤에 의한 니페디핀 생체이용효율 및 약물동태학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 폴리페놀류인 캠페롤을 고혈압치료제인 니페디핀과 병용투여 하였을 때 경구투여된 니페디핀의 생체이용률이 유의성 있게 증가된 것은 캠페롤에 의해서 주로 소장에 존재하는 P-glycoprotein (P-gp) 억제에 의한 흡수증가와 주로 소장과 간장에 존재하는 cytochrome P450 (CYP) 3A4 억제에 의한 니페디핀의 초회통과효과

(대사)감소 또는 전신클리어런스 감소에 기인한 것으로 사료된다.

또한, 니페디핀의 생체이용효율을 유의성 있게(*P < 0.01) 증가시킨 캠페롤이 식품 중에 가장 많이 함유되어 있는 것으로 알려진 양파 120~160 g에 약 10 ㎎정도 함유되어 있으므로, 양파와 니페디핀을 병용투여 할 경우에는 니페디핀의 약물동태에 미치는 영향을 고려하여 용량을 조절하는 것이 바람직하다고 사료된다.

A. Introduction

Nifedipine is predominantly metabolized by CYP3A4 to its primary pyridine metabolite, dehydronifedipine[4, 5]. CYP enzymes are responsible for the oxidative metabolism of many xenobiotics and play a major role in the phase I metabolism of many drugs[6]. CYP3A4 is the most abundant CYP enzyme (30-40%) in adult liver and metabolizes more than 50% of the clinically used drugs including nifedipine, cyclosporine, midazolam, and erythromycin[7, 8].

Nifedipine is a calcium channel-blocking agent that is widely used for the treatment of essential hypertension, coronary artery spasm, and angina pectoris[1]. It inhibits the influx of extracellular calcium through myocardial and vascular membrane pores by physically plugging the channel, resulting in decreased intracellular calcium levels, inhibition of the contractile processes of smooth muscle cells, dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, and decreased total peripheral resistance, systemic blood pressure, and afterload [2, 3].

There are some reports that nifedipine is a substrate of CYP3A4 in human[9-11]. P-gp is an adenosine-50-triphosphate (ATP) dependent efflux drug transporter that is constitutively expressed in normal tissues that includes gastrointestinal epithelium, canalicular membrane of the liver, kidney[12, 13] and capillary endothelial cells in the central nervous system [14, 15]. Because of such tissue localized and its broad substrate specificity, P-gp appears to play a key role in absorption, distribution, and elimination of many drugs[16, 17]. It is generally known that the substrate and/or inhibitors of CYP3A4 and P-gp overlap with each other[18]. Dorababu et al.[19] reported that nifedipine belonged to a group of P-gp substrate. Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically to promote presystemic drug metabolism, resulting in the limited absorption of drugs.

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Kaempferol (3,4',5,7-tetrahydroxyflavone), a flavonoid, is most widely distributed in onion, carrot, and black tea; contents of kaempferol were 832, 140, and 118 mg/kg of dry weight, respectively[25]. Kaempferol is also a CYP3A4 inhibitor[26]. Kaempferol has been reported to be a substrate[27] and an inhibitor[28] of P-gp. But the effect of kaempferol on CYP3A4 and P-gp inhibition is partially ambiguous. Thus, we reevaluated CYP3A4 and P-gp activity using rhodamine-123 retention assay in P-gp overexpressed adriamycin-resistant human breast cancer cell line (MCF-7/ADR).

For example, kaempferol exhibited a remarkable inhibition of P-gp-mediated efflux of ritonavir, thus cellular uptake of ritonavir increased[29]. Thus, it could be expected that kaempferol would change bioavailability and pharmacokinetics of nifedipine by inhibiting P-gp and CYP3A4 in rats.

Furthermore, since kaempferol has become readily available over-the-counter, it is possible that kaempferol products would be taken along with prescribed nifedipine for the therapy of cardiovascular disease. Therefore, the aim of this study was to examine the effects of kaempferol on the CYP3A4, P-gp activity and bioavailability and pharmacokinetics of nifedipine after oral and intravenous administration with kaempferol in rats.

We evaluated CYP enzymes activities and P-gp activity about kaempferol using CYP inhibition assays and rhodamine-123 retention assays in P-gp-over-expressed MCF-7/ADR cells. Kaempferol is expected to change the bioavailability and pharmacokinetics of drugs those are substrates of P-gp and/or CYP3A4, if they are used concomitantly.

Nifedipine and kaempferol interact with CYP enzymes and P-gp, and the increased use of health supplements may result in kaempferol being taken concomitantly with nifedipine to treat or prevent cardiovascular diseases as a combination therapy. It is important to assess the potential bioavailability and pharmacokinetics interactions after the concurrent use of nifedipine and kaempferol or kaempferol containing dietary supplement in order to assure the effectiveness and safety of drug therapy. Antihypertensive agents are commonly co-administered with kaempferol, herbar medicine, for the prevention or treatment of

cardiovascular diseases as a combination therapy in clinics. But there are fewer reports about the effects of herbar medicine, over-the-counter (OTC) medicine, on the bioavailability or pharmacokinetics of nifedipine in rats[39-45].

However, the effect of kaempferol on the bioavailability and pharmacokinetics of nifedipine *in vivo* has not yet been reported. Thus, the purpose of this study is to investigate the possible effects of kaempferol on the CYP3A4 and P-gp activity and bioavailability or the pharmacokinetics of nifedipine and its active metabolite, dehydronifedipine, after oral and intravenous administration of nifedipine with kaempferol in rats.

B. Materials and Methods

1. Materials

Nifedipine, dehydronifedipine, kaempferol and amlodipine [internal standard for the high-performance liquid chromatographic (HPLC) analysis of nifedipine] were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). Methanol, isooctane, methyl-*tert*-butyl ether (MTBE), analytical grade acetic acid and triethylamine (TEA) were purchased from Merck Co. (Darmstadt, Germany). Rhodamine was obtained from Calbiochem (Merck Co., Darmstadt, Germany) and the CYP inhibition assay kit was obtained from GENTEST (Woburn, MA, USA). Other chemicals were of reagent or HPLC grade.

Apparatus used in this study included a HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus auto sampler and a Waters^{TM} 2487 scanning UV detector (Waters Co., Milford, MA, USA), a HPLC column temperature controller (Phenomenex Inc., Torrance, CA, USA), a Bransonic® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., New York, NY, USA), and a high-speed micro-centrifuge (Hitachi Co., Tokyo, Japan).

2. Cell culture

The Michigan Cancer Foundation-7 (MCF-7) cells, human breast carcinoma cell line, and the Michigan Cancer Foundation-7/Adriamycin resistant (MCF-7/ADR) cells, multidrug resistant human breast carcinoma cell line, were obtained from Lab. of Pharmaceutics (College of Pharmacy, Chosun University, Korea). The MCF-7 cells and the MCF-7/ADR cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented 10% (v/v) fetal bovine serum and 100 U/ mL penicillin and 100 μg/ mL streptomycin at 37℃ in an incubator with 5% CO_2 humidified atmosphere.

3. Animal studies

All animal study protocols were approved by the Animal Care Committee of Chosun University (Gwangju, Republic of Korea). Male Sprague-Dawley rats (270-300 g) were purchased from Dae Han Laboratory Animal Research Co. (Eumsung, Republic of Korea), and were given free access to a normal standard chow diet (No. 322-7-1; Superfeed Co., Wonju, Republic of Korea) and tap water. Throughout the experiments, 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 rats were acclimated under these conditions for at least 1 week. Each rat was fasted for at least 24 h prior to the experiment. The left femoral artery (for blood sampling) and left femoral vein (for drug administration in the intravenous study) were cannulated using a polyethylene tube (SP45; i.d., 0.58 mm, o.d., 0.96 mm; Natsume Seisakusho Company, Tokyo, Japan) while each rat was under light.

4. Intravenous and oral administration of nifedipine

The rats were divided into eight groups (n = 6, each): oral groups [10 mg/kg of nifedipine dissolved in distilled water (1.0 mL/kg)] without (control) or with 0.4, 2 and 10 mg/kg of kaempferol (mixed in distilled water; total oral volume of 1.0 mL/kg), and intravenous groups [2.5 mg/kg of nifedipine dissolved in 0.9% Nacl solution; total injection volume of 1.0 mL/kg] without (control) or with 0.4, 2 and 10 mg/kg of kaempferol. A feeding tube was used to administer nifedipine and kaempferol intragastrically. Kaempferol was administered 30 min prior to oral administration of nifedipine. Nifedipine for intravenous administration was injected through the femoral vein within 0.5min. A blood sample (0.5 mL aliquot) was collected into heparinized tubes via the femoral artery at 0.017 (at the end of infusion), 0.1, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h for the intravenous study and 0.25, 0.5, 0.75, 1, 2, 4, 8, 12 and 24 h for the oral study. Approximately 1.2 mL of whole blood collected from untreated rats was infused via the femoral artery at 0.75, 4 and 8 h, respectively, to replace blood loss due to blood sampling. The blood samples were centrifuged (15,300×g, 3 min), and a 200 μL aliquot

of plasma samples was stored in the deep freezer at -40°C until the HPLC analysis.

5. HPLC assay

The plasma concentrations of nifedipine were determined using an HPLC assay by a modification of the method reported by Grundy et al.[46]. Briefly, 50 µL aliquot of 3 µg/mL amlodipine, as the internal standard, and 50 µL of 1.0 M sodium hydroxide were added to 0.2 mL of the plasma sample. It was then mixed for 3 s and 5 mL MTBE-isooctane (75 : 25, v/v) was added. The resultant mixture was vortex-mixed for 1 min and centrifuged at 800×g for 5 min. After centrifugation, the organic layer (0.8 mL) was transferred into a clean test tube and evaporated under a gentle stream of nitrogen gas (no heat applied). The dried extract was reconstituted with 200 µL of mobile, phase vortex-mixed for 1 min and aliquots of 160 µL were transferred to a clean auto sampler vial. A 70 µL aliquot of the supernatant was injected into the HPLC system. The UV detector wavelength was set to 350 nm; and the column, a Nova-pack C_8 (100 mm × 8 mm I.D., 4 µm; Waters Co., Milford, MA, USA), was used at room temperature. The mobile phase consisted of methanol : water (62 : 38, v/v, pH 4.5, adjusted with acetic acid, 320 µL TEA/1000 mL mixture was added). The flow rate of mobile phase was maintained at 1.0 mL/min. Nifedipine, dehydronifedipine and internal standard were eluted with retention times at 8.320, 6.486 and 17.468 min, respectively (Figure 2). The detection limits of nifedipine and dehydronifedipine in rat plasma were all 5 ng/mL (Figure 3, 4). The coefficients of variation for nifedipine and dehydronifedipine were all below 5.0%.

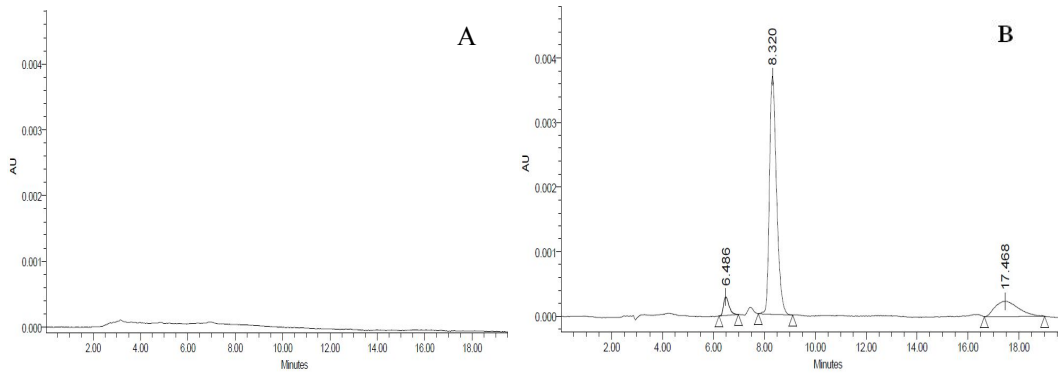

Figure 2. HPLC chromatograms of the rat blank plasma (A) and the plasma spiked with nifedipine (8.320 min), dehydronifedipine (6.486 min) and amlodipine (internal standard; 17.468 min) (B).

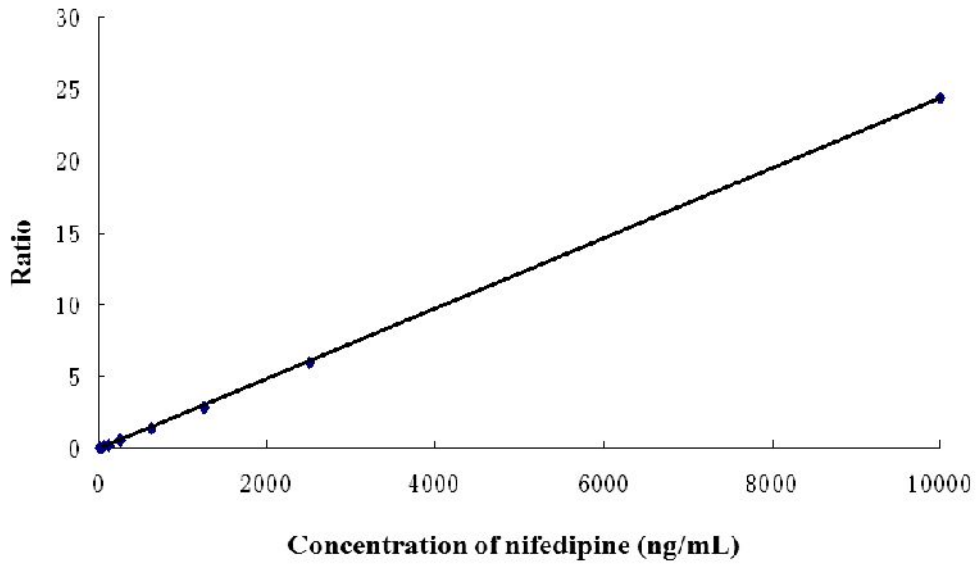

Figure 3. A calibration curve of nifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was y=0.0024x - 0.0983, where "y" is the peak area ratio of nifedipine to amlodipine and "x" is the concentration of nifedipine.

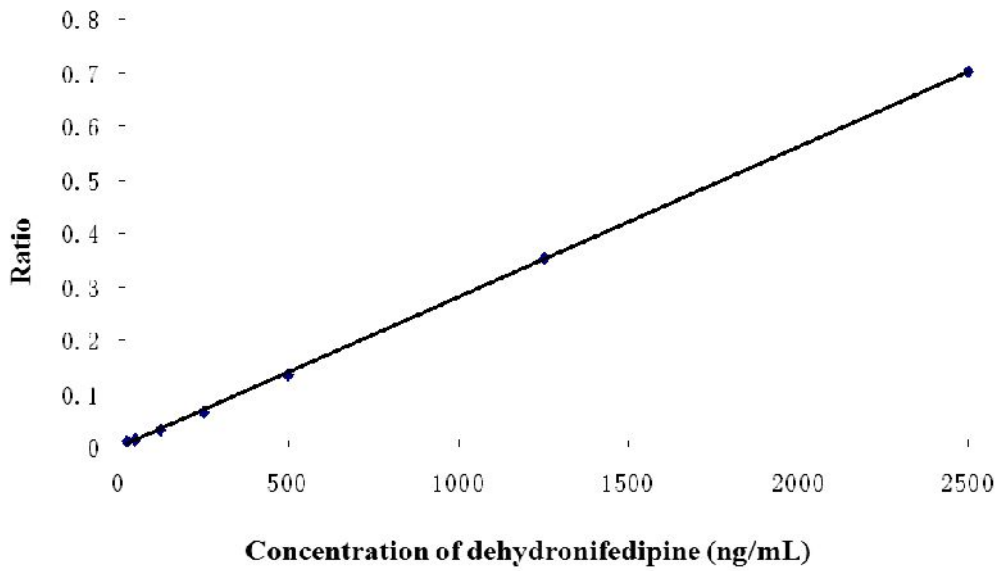

Figure 4. A calibration curve of dehydronifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was $y=0.0003x-0.0014$, where "y" is the peak area ratio of dehydronifedipine to amlodipine and "x" is the concentration of dehydronifedipine.

6. CYP3A4 inhibition assay

The assay of inhibition on human cytochrome P450 (CYP) 3A4 enzyme activity was performed in a multiwell plate using CYP inhibition assay kit (GENTEST, Woburn, MA, USA) as described previously[47]. Briefly, human CYP enzyme was obtained from baculovirus-infected insect cells. CYP substrate (7-BFC for CYP3A4) was incubated with or without kaempferol in the enzyme/substrate buffer consisting of 1 pmol of CYP enzyme and an 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 incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Device, Sunnyvale, CA, USA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 μM ketoconazole for CYP3A4) was run on the same plate and produced 99% inhibition. The results were expressed as the percent of inhibition.

7. Rhodamine-123 retention assay

The procedures used for the Rho-123 retention assay were similar to a reported method [48]. The MCF-7 cells and the MCF-7/ADR cells, the P-glycoprotein (P-gp)-overexpressed multidrug resistant human breast carcinoma cell line, were seeded in 24-well plates. At 80% confluence, the cells were incubated in FBS-free DMEM for 18 h. The culture medium was changed to Hanks' balanced salt solution and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20 μM rhodamine-123 in the presence or absence of kaempferol (50 and 100 μM) and verapamil (positive control, 100 μM) 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 EBC lysis buffer.

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 were presented as the ratio to control.

8. Bioavailability and pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using Thermo Kinetica Software Version 5.0 (Thermo Fisher Scientific Inc., Miami, OK, USA). The parameter values were obtained by fitting to the pharmacokinetic model using the simplex algorithm. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) was calculated by a trapezoidal rule. The peak concentration (C_{max}) of nifedipine in plasma and time to reach C_{max} (T_{max}) were obtained by visual inspection of the data from the concentration–time curve. The terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. Total body clearance (CL/F) was calculated by dose/AUC. The absolute bioavailability (AB) was calculated by $AUC_{oral}/AUC_{i.v.} \times dose_{i.v.}/dose_{oral}$, and the relative bioavailability (RB) of nifedipine were calculated by $AUC_{nifedipine\ with\ kaempferol}/AUC_{control}$. The metabolite–parent AUC ratio (MR) was calculated by $AUC_{dehydronifedipine}/AUC_{nifedipine}$.

9. Statistical analysis

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

C. Results

1. Inhibition of CYP3A4

The inhibitory effect of kaempferol on CYP3A4 activity is shown in Figure 5. Kaempferol inhibited CYP3A4 activity in a concentration-dependent manner. Kaempferol inhibited CYP3A4 activity with an IC_{50} value of 9.8 μM.

2. Rhodamine-123 retention assay

The effect of kaempferol on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cell is shown in Figure 6. Accumulation of rhodamine-123, a P-gp substrate, was decreased in MCF-7/ADR cells overexpressing P-gp compared to that in MCF-7 cells lacking P-gp. The concurrent use of kaempferol ($P < 0.05$ for 100 μM) enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner. This result suggests that kaempferol significantly inhibited P-gp activity.

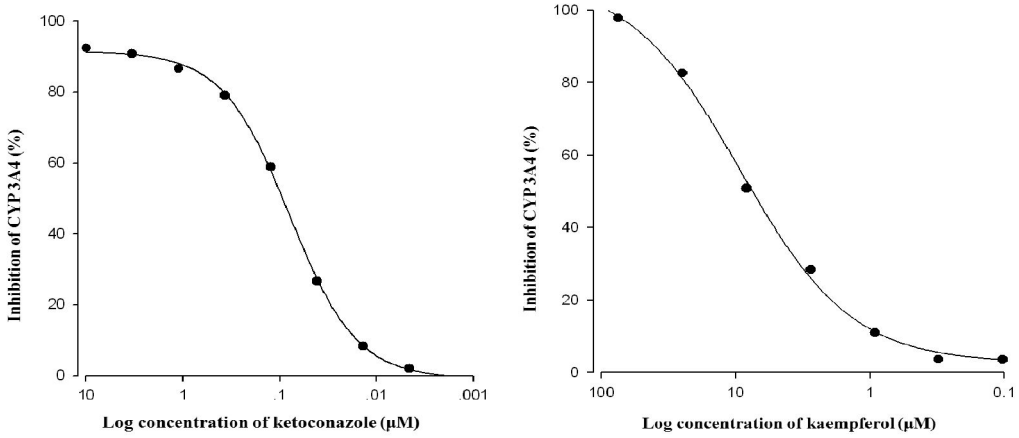

Figure 5. Inhibitory effect of ketoconazole and kaempferol on CYP3A4 activity. The results were expressed as the percent of inhibition.

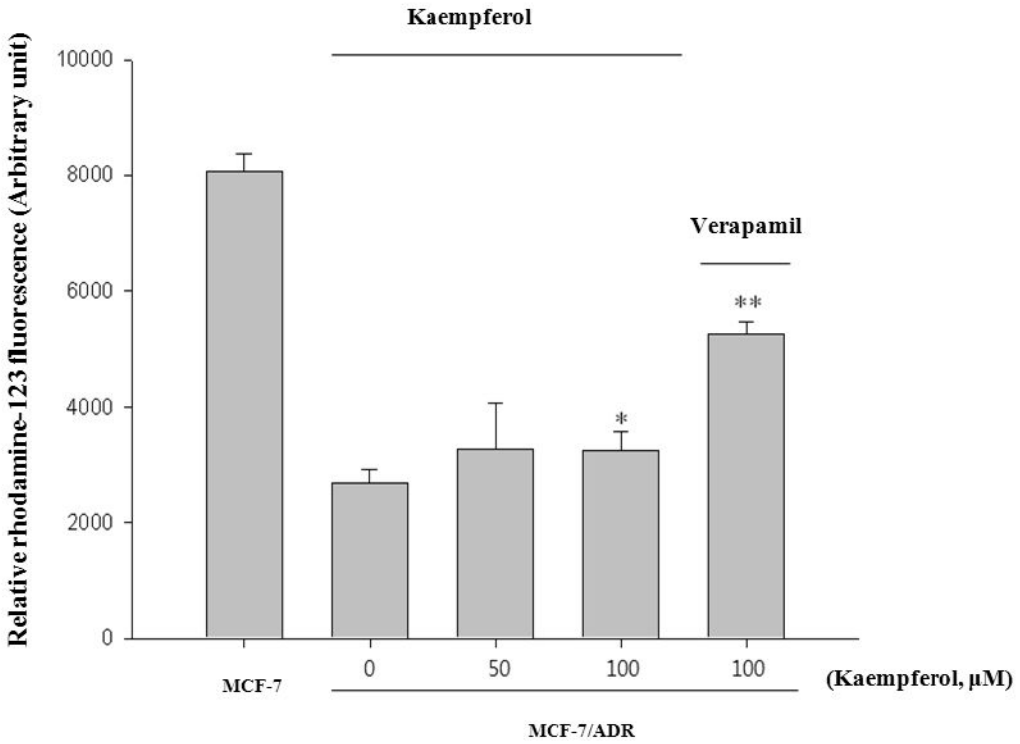

Figure 6**.** Effects of kaempferol on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD (n = 6).

* $P < 0.05$, ** $P < 0.01$, significant difference compared to positive control (Verapamil).

3. Effect of kaempferol on the bioavailability of oral nifedipine

The mean plasma concentration-time profiles of nifedipine in the presence and absence of kaempferol (0.4, 2 and 10 mg/kg) are shown in Figure 7. The bioavailability and pharmacokinetic parameters of nifedipine are summarized in Table 1. Kaempferol significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased the area under the plasma concentration-time curve from time zero to time infinity ($AUC_{0-\infty}$) of nifedipine by 29.3-45.4%, and peak concentration (C_{max}) of nifedipine by 41.8%. The total body clearance (CL/F) was significantly (10 mg/kg, $P < 0.05$) decreased (22.8-31.3%) by kaempferol. Accordingly, the absolute bioavailability (AB) values of nifedipine in the presence of kaempferol were significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) higher (28.5-44.9%) than that of the control group. Kaempferol increased the relative bioavailability (RB) of nifedipine by 1.29- to 1.45-fold. There were no significant differences in the time to reach peak plasma concentration (T_{max}), terminal half-life ($t_{1/2}$) of nifedipine in the presence of kaempferol.

4. Effect of kaempferol on the bioavailability of dehydronifedipine

The plasma concentration-time profiles of dehydronifedipine are shown in Figure 8. The bioavailability and pharmacokinetic parameters of dehydronifedipine are summarized in Table 2. The Kaempferol significantly (10 mg/kg, $P < 0.05$) increased the area under the plasma concentration-time curve from time zero to time infinity ($AUC_{0-\infty}$) of dehydronifedipine by 25.7%. The metabolite-parent AUC ratios (MR) were significantly ($P < 0.05$, 10 mg/kg) decreased by kaempferol, suggesting that the formation of dehydronifedipine was considerably altered by kaempferol. Thus, the increased bioavailability of nifedipine by kaempferol may be mainly due both to the inhibition of P-gp activity in the small intestine and to the inhibition of CYP3A4 activity in the small intestine and/or in the liver by kaempferol.

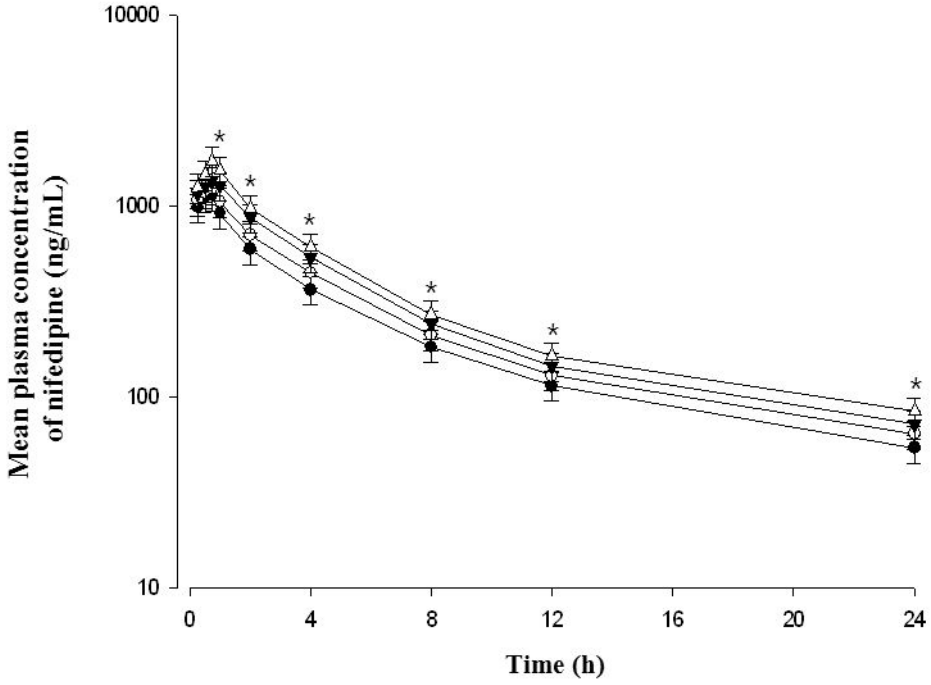

Figure 7**.** Mean plasma concentration-time profiles of nifedipine after oral administration of nifedipine (10 mg/kg) to rats in the presence and absence of kaempferol (0.4, 2 or 10 mg/kg). (mean ± SD, n = 6). (●) nifedipine alone, (○) with 0.4 mg/kg kaempferol, (▼) with 2 mg/kg kaempferol, (△) with 10 mg/kg kaempferol.

* $P < 0.01$, significant difference compared to the control group given nifedipine alone.

Table 1. Mean bioavailability and pharmacokinetic parameters of nifedipine after oral administration of nifedipine (10 mg/kg) to rats in the presence or absence of kaempferol

Parameters	**Nifedipine (control)**	**Nifedipine + Kaempferol**		
		0.4 mg/kg	2 mg/kg	10 mg/kg
$AUC_{0-\infty}$ (ng h/mL)	5930 ± 1067	6832 ± 1229	7668 ± 1304*	8622 ± 1465**
C_{max} (ng/mL)	1130 ± 192	1199 ± 215	1298 ± 220	1602 ± 277*
T_{max} (h)	0.71 ± 0.19	0.71 ± 0.19	0.69 ± 0.14	0.69 ± 0.14
CL/F (mL/hr/kg)	28.1 ± 5.1	24.4 ± 4.4	21.7 ± 3.4*	19.3 ± 3.2**
$t_{1/2}$ (h)	9.5 ± 1.8	9.8 ± 1.9	10.0 ± 2.0	10.1 ± 2.1
AB (%)	15.8 ± 2.7	18.2 ± 3.5	20.3 ± 3.5*	22.9 ± 3.9**
RB (%)	100	115	129	145

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

(mean ± SD, $n = 6$).

* $P < 0.05$, ** $P < 0.01$, significant difference compared to the control group given nifedipine alone.

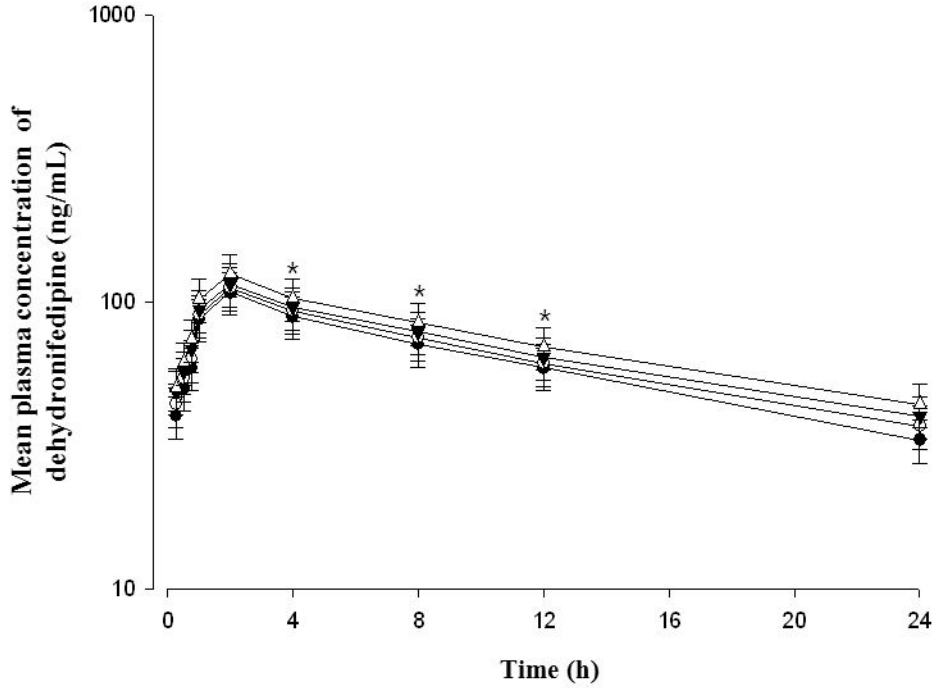

Figure 8. Mean plasma concentration-time profiles of dehydronifedipine after oral administration of nifedipine (10 mg/kg) to rats in the presence and absence of kaempferol (0.4, 2 or 10 mg/kg).

(mean ± SD, n = 6). (●) nifedipine alone, (○) with 0.4 mg/kg kaempferol, (▼) with 2 mg/kg kaempferol, (△) with 10 mg/kg kaempferol.

* $P < 0.05$, significant difference compared to the control group given nifedipine alone.

Table 2. Mean bioavailability and pharmacokinetic parameters of dehydronifedipine after oral administration of nifedipine (10 mg/kg) to rats in the presence or absence of kaempferol

Parameters	**Nifedipine (control)**	**Nifedipine+ Kaempferol**		
		0.4 mg/kg	2 mg/kg	10 mg/kg
$AUC_{0-\infty}$ (ng h/mL)	2144 ± 385	2337 ± 381	2463 ± 428	2694 ± 451*
C_{max} (ng/mL)	108 ± 19	109 ± 20	112 ± 20	118 ± 22
T_{max} (h)	2.00 ± 1.09	2.00 ± 1.09	2.17 ± 0.98	2.17 ± 0.98
$t_{1/2}$ (h)	14.4 ± 2.5	15.9 ± 2.6	16.2 ± 2.9	17.0 ± 3.0
AB (%)	100	109	114	126
MR (%)	0.36 ± 0.05	0.34 ± 0.06	0.32 ± 0.05	0.31 ± 0.04*

$AUC_{0-\infty}$, 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; RB, relative bioavailability; MR, metabolite-parent ratio ($AUC_{dehydronifedipine}/AUC_{nifedipine}$).

(mean ± SD, $n = 6$).

* $P < 0.05$, significant difference compared to the control group given nifedipine alone.

5. Effect of kaempferol on the bioavailability of intravenous nifedipine

Mean arterial plasma concentration-time profiles of nifedipine following an intravenous administration of nifedipine (2.5 mg/kg) to rats in the presence or absence of kaempferol (2 and 10 mg/kg) are shown in Figure 9, while the corresponding bioavailability and pharmacokinetic parameters are shown in Table 3. The $AUC_{0-\infty}$ and CL_t of nifedipine were changed, but was not statistically significant compared to those in the control. The $t_{1/2}$ of nifedipine was also prolonged, but this increase was not statistically significant. The bioavailability and pharmacokinetic of intravenous nifedipine was not affected by the concurrent use of kaempferol in contrast to those of oral nifedipine.

Accordingly, the enhanced oral bioavailability in the presence of kaempferol, while there was no significant change in the bioavailability and pharmacokinetics of intravenous nifedipine, may be mainly due to inhibition of the CYP3A-mediated metabolism of nifedipine in the small intestine and/or in the liver by kaempferol rather than renal elimination of nifedipine.

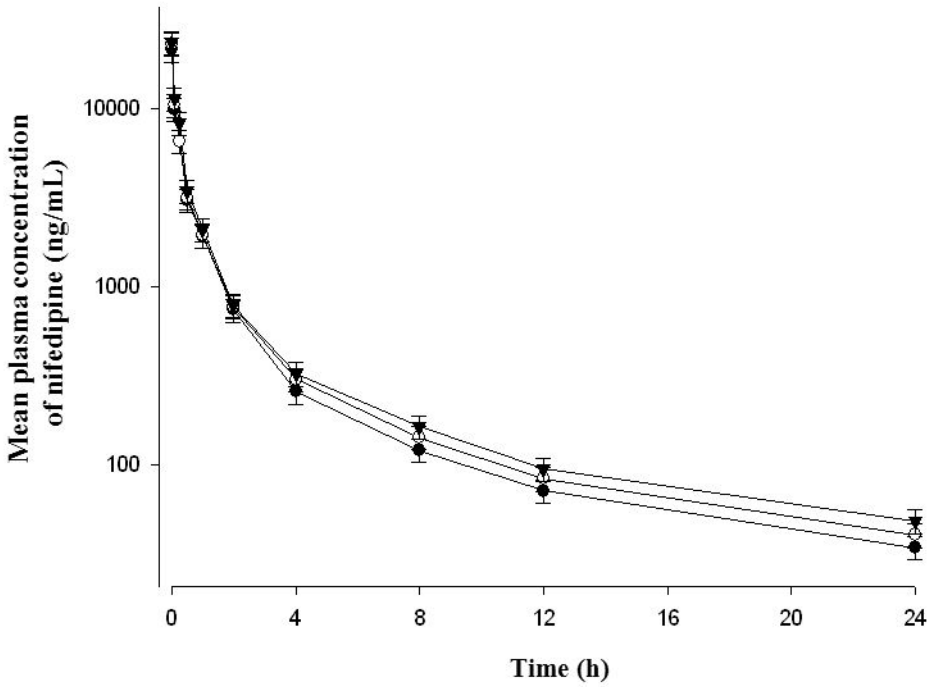

Figure 9. Mean plasma concentration-time profiles of nifedipine after intravenous administration of nifedipine (2.5 mg/kg) to rats in the presence and absence of kaempferol (2 or 10 mg/kg).

(mean ± SD, *n* = 6). (●) nifedipine alone, (○) with 2 mg/kg kaempferol, (▼) with 10 mg/kg kaempferol.

Table 3. Mean bioavailability and pharmacokinetic parameters of nifedipine after intravenous (2.5 mg/kg) administration to rats in the presence or absence of kaempferol

Parameters	**Nifedipine (control)**	**Nifedipine + Kaempferol**	
		2 mg/kg	10 mg/kg
$AUC_{0-\infty}$ (ng h/mL)	9412 ± 1693	9992 ± 1753	10978 ± 1876
CL_t (mL/hr/kg)	9.2 ± 1.6	8.9 ± 1.5	8.7 ± 1.5
$t_{1/2}$ (h)	9.2 ± 1.7	9.3 ± 1.8	9.7 ± 1.9
RB (%)	100	106	116

$AUC_{0-\infty}$, total area under the plasma concentration–time curve from time zero to infinity; CL_t, total body clearance; $t_{1/2}$, terminal half-life; RB, relative bioavailability.

(mean ± SD, n = 6).

D. Discussion

Based on the broad overlap in substrate specificities, as well as their co-localization in the small intestine as the primary site of absorption for orally administered drugs, CYP3A4 and P-gp are recognized as a concerted barrier to drug absorption[49]. CYP enzymes significantly contribute to the first-pass metabolism and the oral bioavailability. Moreover, induction or inhibition of intestinal CYP enzymes may be responsible for significant drug-drug interactions[50], in when one agent decreases or increases the bioavailability and absorption rate constant of another drug administered concurrently[51]. Therefore, dual inhibitors against both CYP3A4 and P-gp should have a great impact on the bioavailability of many drugs where CYP3A4 metabolism and P-gp mediated efflux is the major barrier to the systemic availability.

With the great interest in herbal components as alternative medicines, much effort is currently being expended to identify natural compounds of plant origin that modulate P-gp and metabolic enzymes, however, there is far less information on the pharmacokinetic interactions between herbal components and medicines. More preclinical and clinical investigations on the herbal constituents-drug interaction should be performed to prevent potential adverse reactions in patients or to utilize those interactions for a therapeutic benefit. Therefore, the present study evaluated the effect of kaempferol, a naturally occurring flavonoid, on the bioavailability and pharmacokinetics of nifedipine in rats to examine a potential drug interaction between kaempferol and nifedipine via the dual inhibition of CYP3A4 and P-gp.

As shown in Figures 5 and 6, kaempferol exhibited an inhibitory effect against CYP3A4 and significantly ($P < 0.05$) inhibited P-gp activity. These results are consistent with the previous report [26, 28].

Those results suggest that kaempferol might be effective to improve the bioavailability of nifedipine, a substrate of CYP3A4 and P-gp. Therefore, the bioavailability of characteristics of nifedipine were evaluated in the absence and the presence of kaempferol in rats. As CYP3A4 expressed in rat is similar and corresponding to the action of CYP3A4 in human[40, 43-45, 52].

It is possible that the concomitant administration of kaempferol might affect the bioavailability and pharmacokinetics of orally administered nifedipine. Since orally administered nifedipine is a substrate for CYP3A4-mediated metabolism and P-gp-mediated efflux in the intestine and in liver.

As summarized in Table 1, kaempferol significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased $AUC_{0-\infty}$ of nifedipine by 29.3-45.4% and C_{max} of nifedipine by 41.8%. The total body clearance (CL/F) was significantly decreased (22.8-31.3%) (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) by kaempferol. The absolute bioavailability (AB) of nifedipine in the presence of kaempferol was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) higher (28.5-44.9%) than that in the control group.

As summarized in Table 2, kaempferol significantly decreased metabolite-parent AUC ratios (MR). In the present study, therefore, the decrease in the metabolite-parent AUC ratios (MR) of nifedipine might be mainly due to the inhibitory effect of kaempferol on the first-pass effects (metabolism) in the liver and/or in the small intestine. This result is coincident with the previous study, that the presence of kaempferol significantly increased AUC of tamoxifen [53] and that kaempferol significantly increased AUC of etoposide[54].

Kaempferol did not significantly change the bioavailability and pharmacokinetic parameters of intravenous administration of nifedipine (Table 3), suggesting that kaempferol may improve the oral bioavailability of nifedipine by more increasing the absorption or reducing intestinal metabolism of nifedipine by the inhibition of CYP3A rather than renal elimination by kaempferol.

Therefore, the enhanced bioavailability of nifedipine might be mainly due to inhibition of the CYP3A-mediated metabolism of nifedipine in the small intestine and/or in the liver and inhibition of the P-gp efflux transporter in the small intestine by kaempferol.

Although potential adverse effects, this interaction may provide a therapeutic benefit whereby it enhances bioavailability and lowers the dose administered. Since the present study raised the awareness about the potential drug interactions by concomitant use of kaempferol, a natural flavonoid, with nifedipine, the clinical significance of this finding need to be further

evaluated in the clinical studies.

In contrast, kaempferol had no effect on any bioavailability and pharmacokinetic parameters of nifedipine given intravenously implying that coadministration of a kaempferol could inhibit CYP3A4-mediated metabolism and P-gp-mediated efflux of nifedipine, resulting in reducing intestinal or hepatic first-pass metabolism.

Kaempferol significantly enhanced the oral bioavailability of nifedipine, which might be primarily attributable to the promotion of intestinal absorption and reduction of first-pass metabolism of nifedipine in the intestinal and/or liver via inhibition of P-gp and CYP3A4 by kaempferol.

It is reported that kaempferol contains 677~832 mg/kg of dry weight in onion and 2.4~132.0, 13-30 and16-19 mg/kg of fresh weight in broccoli, kale and gooseberry, respectively [25, 55-58].

It is expected that intake of food such as approximately 120~160 g of onion, 80~400 g of broccoli, 330-650 g of kale or 520-600 g of gooseberry corresponding to 10 mg of kaempferol can significantly increase the bioavailability of nifedipine. Therefore, the dose of nifedipine should be reduced when coadministered with kaempferol for rational dosage regimen.

E. Conclusion

The increased bioavailability of nifedipine in the presence of kaempferol might be due to an inhibition of the P-gp-mediated efflux transporter in the small intestine and to an inhibition of CYP 3A-mediated metabolism in the small intestine and/or in the liver rather than to a reduction of renal elimination by kaempferol.

Concomitant use of nifedipine with kaempferol may require close monitoring for potential drug interactions. However, the clinical importance of these findings should be further investigated in clinical trials.

F. References

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Abstract

The purpose of this study was to investigate the possible effects of kaempferol on the bioavailability and pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, in rats. The bioavailability or pharmacokinetic parameters of nifedipine and dehydronifedipine were determined after oral and intravenous administration of nifedipine to rats in the presence and absence of kaempferol (0.4, 2 and 10 mg/kg).

The effect of kaempferol on P-glycoprotein (P-gp) and cytochrome P450 (CYP) 3A4 activity was evaluated. Kaempferol inhibited CYP3A4 enzyme activity in a concentration-dependent manner with a 50% inhibition concentration (IC_{50}) of 9.8 μM. In addition, kaempferol significantly enhanced the cellular accumulation of rhodamine-123 in MCF-7/ADR cells overexpressing P-glycoprotein.

The bioavailability and pharmacokinetic parameter of nifedipine were significantly changed by the presence of kaempferol compared with the control group. The areas under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak concentration (C_{max}) of nifedipine were significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased by 29.3-45.4% and 41.8%, respectively, with kaempferol. The total body clearance (CL/F) was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) decreased by kaempferol (22.8-31.3%). Consequently, the absolute bioavailability (AB) of nifedipine in the presence of kaempferol was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) higher (28.5-44.9%) than that of the control group. Moreover, the relative bioavailability (RB) of nifedipine was 1.29- to 1.45-fold greater than that in the control group. The metabolite-parent AUC ratio (MR) in the presence of kaempferol (10 mg/kg) significantly ($P < 0.05$) decreased compared to the control group. This result implied that coadministration of kaempferol could be effective to inhibit the metabolism of nifedipine.

In conclusion, the increased bioavailability of nifedipine in the presence of kaempferol might be due to an inhibition of the P-gp-mediated efflux transporter in the small intestine and CYP3A-mediated metabolism in the small intestine and/or in the liver or to a reduction of total body clearance rather than to a reduction of renal elimination of nifedipine by kaempferol.

Key words: Nifedipine; dehydronifedipine; kaempferol; CYP3A4; P-gp; pharmacokinetics; bioavailability; rats

Part II. Effects of licochalcone A on the bioavailability of nifedipine in rats

국 문 초 록

한방에서 빈용되며 비처방약인 감초성분인 리코찰콘 A와 고혈압약물로 빈용되는 니페디핀의 병용투여되는 경우가 가능하므로 이에 대한 상호작용을 알아보고자, 흰쥐에 니페디핀 (경구; 10 mg/kg, 정맥; 2.5 mg/kg)과 리코찰콘 A (0.4, 2, 10 mg/kg)를 병용투여한 후 니페디핀 및 주 대사체인 디히드로니페디핀의 생체이용효율 및 약물동태학적 변수들을 대조군과 비교 검토하였다.

리코찰콘 A는 cytochrome P450 (CYP) 3A4 억제작용과 P-glycoprotein (P-gp) 억제 작용이 있었다. 리코찰콘 A와 병용 투여 시 니페디핀의 약물동태학적 변수는 유의성 있게 변화하였다. 대조군에 비해 리코찰콘 A (2, 10 mg/kg)와 병용투여군에서 니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 각각 유의성 ($P < 0.05$, $P < 0.01$) 있게 증가되었으며, 전신클리어런스 (CL/F)는 유의성 ($P < 0.05$, $P < 0.01$) 있게 각각 감소되었다.

절대적 생체이용률 (AB)도 대조군에 비해 각각 유의성 ($P < 0.05$, $P < 0.01$) 있게 증가되었다. 리코찰콘 A (10 mg/kg)와 니페디핀을 병용투여한군에서 대조군에 비해 주대사체인 디히드로니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)이 유의성 ($P < 0.05$) 있게 증가되었다. 그리고 리코찰콘 A는 니페디핀의 대사율 (MR)을 유의성 있게 감소시켰다.

정맥투여군에서는 리코찰콘 A가 니페디핀의 생체이용효율 및 약동학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 폴리페놀인 리코찰콘 A를 고혈압 치료제인 니페디핀과 병용투여

하였을 때 경구 투여된 니페디핀의 생체이용률이 유의성 있게 증가된 것은 리코찰콘 A에 의해서 주로 소장에 존재하는 P-gp억제에 의한 흡수증가와 주로 간장과 소장에 존재하는 CYP3A 억제에 의한 니페디핀의 초회통과효과 (대사) 감소와 전신클리어런스 감소에 기인한 것으로 사료된다.

A. Introduction

Nifedipine, a dihydropyridine calcium channel-blocker, is effective for the treatment of essential hypertension, coronary artery spasm, and angina pectoris[1]. It inhibits the influx of extracellular calcium through myocardial and vascular membrane pores by physically plugging the channel, resulting in decreased intracellular calcium levels, inhibition of the contractile processes of smooth muscle cells, dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, and decreased total peripheral resistance, systemic blood pressure, and afterload[2,3].

Nifedipine is predominantly metabolized by cytochrome P450 (CYP) 3A4 to its primary pyridine metabolite, dehydronifedipine[4,5]. CYP enzymes are responsible for the oxidative metabolism of many xenobiotics and play a major role in the phase I metabolism of many drugs[6]. CYP3A4 is the most abundant CYP enzyme (30-40%) in adult liver and metabolizes more than 50% of the clinically used drugs including nifedipine, cyclosporine, midazolam, and erythromycin[7,8]. There are some reports that nifedipine is a substrate of CYP3A4 in human[9-11]. P-gp is an adenosine-50-triphosphate (ATP) dependent efflux drug transporter that is constitutively expressed in normal tissues that includes gastrointestinal epithelium, canalicular membrane of the liver, kidney[12,13] and capillary endothelial cells in the central nervous system[14,15]. Because of such tissue localized and its broad substrate specificity, P-gp appears to play a key role in absorption, distribution, and elimination of many drugs[16,17]. It is generally known that the substrate and/or inhibitors of CYP3A4 and P-gp overlap with each other[18]. Dorababu et al.[19] reported that nifedipine belonged to a group of P-gp substrate. Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically to promote presystemic drug metabolism, resulting in the limited absorption of drugs.

Flavonoids represent a group of phytochemicals that are produced in high quantities by various plants[20]. These compounds exhibit a wide range of beneficial biological activities including antioxidative, radical-scavenging, anti-atherosclerotic, antitumor and antiviral effects[21]. Licochalcone A is an estrogenic flavonoid and the main active compound of the

licorice species *Glycyrrhiza inflate*[22]. Licochalcone A also has a wide range of biological and pharmacological activities, including antioxidant, superoxide scavenging[23], anti-leishmanial activity, and effects on the function of parasite mitochondria[24], antimalarial activities both *in vitro* and *in vivo*[25], and antitumor activities in cancer cells[26-28]. Kwon *et al*. demonstrated that licochalcone A exerts anti-inflammatory effects by suppressing nuclear factor-kB (NF-kB) and activator protein-1 (AP-1) signaling[29]. Kim *et al*. provide the first evidence that licochalcone A could inhibit the angiogenesis *in vitro* and *in vivo* and the tumor growth[30].

We evaluated CYP enzymes activities and P-gp activity about licochalcone A using CYP inhibition assays and rhodamine-123 retention assays in P-gp-over-expressed MCF-7/ADR cells. Licochalcone A is expected to change the bioavailability and pharmacokinetics of drugs those are substrates of P-gp and/or CYP3A4, if they are used concomitantly.

Nifedipine and licochalcone A interact with CYP enzymes and P-gp, and the increased use of health supplements may result in licochalcone A being taken concomitantly with nifedipine to treat or prevent cardiovascular diseases as a combination therapy. It is important to assess the potential pharmacokinetic interactions after the concurrent use of nifedipine and licochalcone A or licochalcone A containing dietary supplement in order to assure the effectiveness and safety of drug therapy. Antihypertensive agents are commonly co-administered with licochalcone A, herbar medicine, for the prevention or treatment of cardiovascular diseases as a combination therapy in clinics. But there are fewer reports about the effects of herbar medicine, over-the-counter (OTC) medicine, on the bioavailability or pharmacokinetics of nifedipine in rats[31-37].

However, the effect of licochalcone A on the bioavailability and pharmacokinetics of nifedipine has not yet been reported. Thus, the purpose of this study was to investigate the possible effects of licochalcone A on the CYP3A4 and P-gp activity and bioavailability or the pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, after oral and intravenous administration of nifedipine with licochalcone A in rats.

B. Materials and Methods

1. Materials

Nifedipine, dehydronifedipine and amlodipine [internal standard for the high-performance liquid chromatographic (HPLC) analysis of nifedipine] were purchased from the Sigma-Aldrich Co. (St. Louis, MO, USA). Licochalcone A was obtained from Lab. of Natural products Chemistry (College of Pharmacy, Chosun University, Korea). Methanol, isooctane, methyl-*tert*-butyl ether (MTBE), analytical grade acetic acid and triethylamine (TEA) were purchased from Merck Co. (Darmstadt, Germany). Rhodamine was obtained from Calbiochem (Merck Co., Darmstadt, Germany) and the CYP inhibition assay kit was obtained from GENTEST (Woburn, MA, USA). Other chemicals were of reagent or HPLC grade.

Apparatus used in this study included a HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus auto sampler and a Waters™ 2487 scanning UV detector (Waters Co., Milford, MA, USA), a HPLC column temperature controller (Phenomenex Inc., Torrance, CA, USA), a Bransonic® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., New York, NY, USA), and a high-speed micro-centrifuge (Hitachi Co., Tokyo, Japan).

2. Cell culture

The Michigan Cancer Foundation-7 (MCF-7) cells, human breast carcinoma cell line, and the Michigan Cancer Foundation-7/Adriamycin resistant (MCF-7/ADR) cells, multidrug resistant human breast carcinoma cell line, were obtained from Lab. of Pharmaceutics (College of Pharmacy, Chosun University, Korea). The MCF-7 cells and the MCF-7/ADR cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented 10% (v/v) fetal bovine serum and 100 U/ mL penicillin and 100 μg/ mL streptomycin at 37℃ in an incubator with 5% CO_2 humidified atmosphere.

3. Animal studies

All animal study protocols were approved by the Animal Care Committee of Chosun University (Gwangju, Republic of Korea). Male Sprague-Dawley rats (270-300 g) were purchased from Dae Han Laboratory Animal Research Co. (Eumsung, Republic of Korea), and were given free access to a normal standard chow diet (No. 322-7-1; Superfeed Co., Wonju, Republic of Korea) and tap water. Throughout the experiments, 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 rats were acclimated under these conditions for at least 1 week. Each rat was fasted for at least 24 h prior to the experiment. The left femoral artery (for blood sampling) and left femoral vein (for drug administration in the intravenous study) were cannulated using a polyethylene tube (SP45; i.d., 0.58 mm, o.d., 0.96 mm; Natsume Seisakusho Company, Tokyo, Japan) while each rat was under light.

4. Intravenous and oral administration of nifedipine

The rats were divided into eight groups (n = 6, each): oral groups [10 mg/kg of nifedipine dissolved in distilled water (1.0 mL/kg)] without (control) or with 0.4, 2 and 10 mg/kg of licochalcone A (mixed in distilled water; total oral volume of 1.0 mL/kg), and intravenous groups [2.5 mg/kg of nifedipine dissolved in 0.9% Nacl solution; total injection volume of 1.0 mL/kg] without (control) or with 0.4, 2 and 10 mg/kg of licochalcone A. A feeding tube was used to administer nifedipine and licochalcone A intragastrically. Licochalcone A was administered 30 min prior to oral administration of nifedipine. Nifedipine for intravenous administration was injected through the femoral vein within 0.5min. A blood sample (0.5 mL aliquot) was collected into heparinized tubes via the femoral artery at 0.017 (at the end of infusion), 0.1, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h for the intravenous study and 0.25, 0.5, 0.75, 1, 2, 4, 8, 12 and 24 h for the oral study. Whole blood (approximately 1.2 mL) collected from untreated rats was infused via the femoral artery at 0.75, 4 and 8 h, respectively, to replace blood loss due to blood sampling. The blood samples were centrifuged (15,300×g, 3 min), and

a 200 μL aliquot of plasma samples was stored in the deep freezer at -40°C until the HPLC analysis.

5. HPLC assay

The plasma concentrations of nifedipine were determined using an HPLC assay by a modification of the method reported by Grundy et al.[38]. Briefly, 50 μL aliquot of 3 μg/mL amlodipine, as the internal standard and 50 μL of 1.0 M sodium hydroxide were added to 0.2 mL of the plasma sample. It was then mixed for 3 s and 5 mL MTBE isooctane (75 : 25, v/v) was added. The resultant mixture was vortex mixed for 1 min and centrifuged at 800×g for 5 min. After centrifugation, the organic layer (0.8 mL) was transferred into a clean test tube and evaporated under a gentle stream of nitrogen gas (no heat applied). The dried extract was reconstituted with 200 μL of mobile phase vortex mixed for 1 min and aliquots of 160 μL were transferred to a clean auto sampler vial. A 70 μL aliquot of the supernatant was injected into the HPLC system. The UV detector wavelength was set to 350 nm; and the column, a Nova-pack C_8 (100mm × 8 mm I.D., 4 μm; Waters Co., Milford, MA, USA), was used at room temperature. A mixture of methanol : water (62 : 38, v/v, pH 4.5, adjusted with acetic acid, 320 μL TEA/1000 mL mixture was added) was used as the mobile phase at a flow rate of 1.0 mL/min. Nifedipine, dehydronifedipine and internal standard were eluted with retention times at 8.320, 6.486 and 17.468 min, respectively (Figure 10). The detection limits of nifedipine and dehydronifedipine in rat plasma were all 5 ng/ mL (Figure 11, 12). The coefficients of variation for nifedipine and dehydronifedipine were all below 5.0.

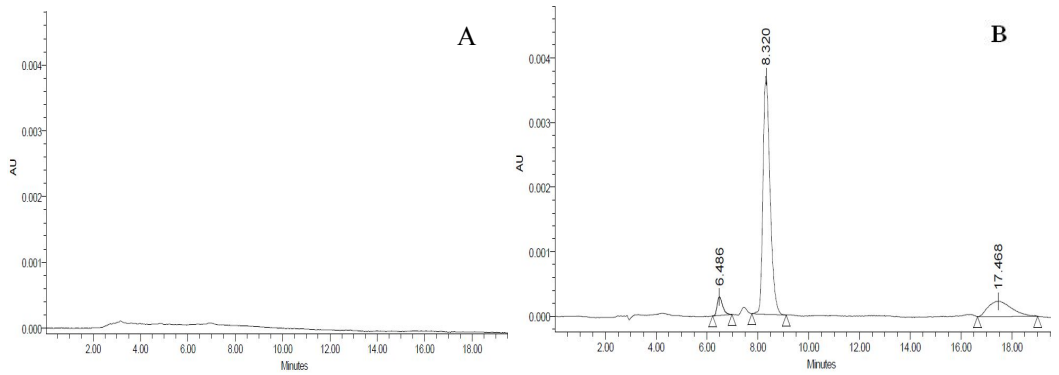

Figure 10. HPLC chromatograms of the rat blank plasma (A) and the plasma spiked with nifedipine (8.320 min), dehydronifedipine (6.486 min) and amlodipine (internal standard; 17.468 min) (B).

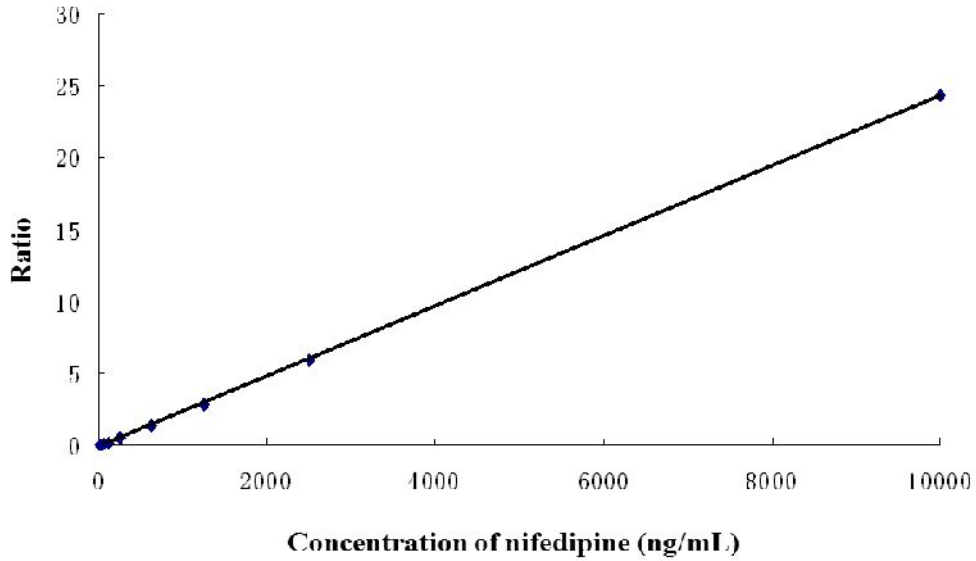

Figure 11. A calibration curve of nifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was y=0.0024x - 0.0983, where "y" is the peak area ratio of nifedipine to amlodipine and "x" is the concentration of nifedipine.

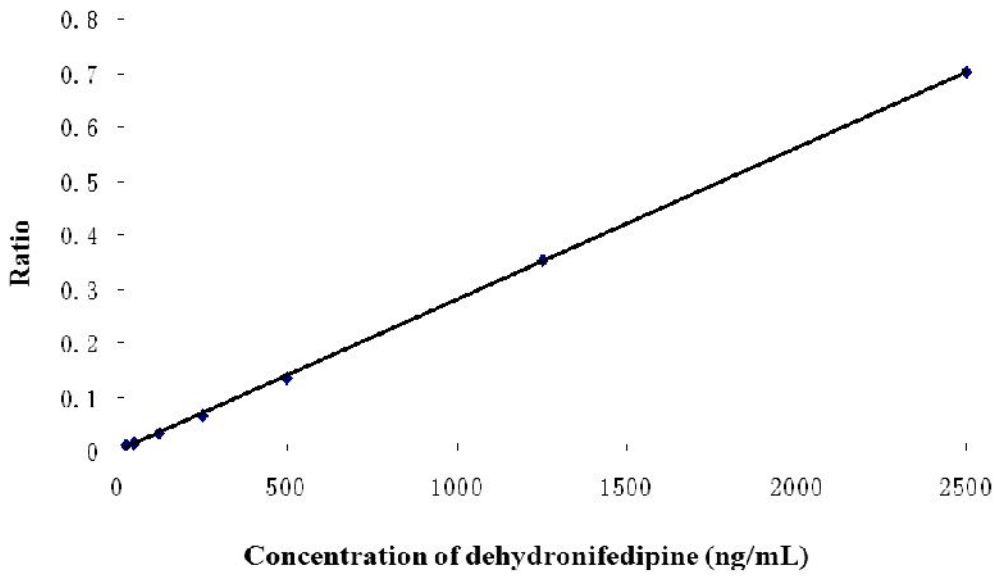

Figure 12. A calibration curve of dehydronifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was $y=0.0003x-0.0014$, where "y" is the peak area ratio of dehydronifedipine to amlodipine and "x" is the concentration of dehydronifedipine.

6. CYP3A4 inhibition assay

The assay of inhibition on human cytochrome P450 (CYP) 3A4 enzyme activity was performed in a multiwell plate using CYP inhibition assay kit (GENTEST, Woburn, MA, USA) as described previously.[39] Briefly, human CYP enzyme was obtained from baculovirus-infected insect cells. CYP substrate (7-BFC for CYP3A4) was incubated with or without licochalcone A in the enzyme/substrate buffer consisting of 1 pmol of P450 enzyme and an 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 incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Device, Sunnyvale, CA, USA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 μM ketoconazole for CYP3A4) was run on the same plate and produced 99% inhibition. The results were expressed as the percent of inhibition.

7. Rhodamine-123 retention assay

The procedures used for the Rho-123 retention assay were similar to a reported method[40]. The MCF-7 cells and the MCF-7/ADR cells, the P-glycoprotein (P-gp)-overexpressed multidrug resistant human breast carcinoma cell line, were seeded in 24-well plates. At 80% confluence, the cells were incubated in FBS-free DMEM for 18 h. The culture medium was changed to Hanks' balanced salt solution and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20 μM rhodamine-123 in the presence or absence of licochalcone A (1, 3 and 10 μM) and verapamil (positive control, 100 μM) 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 EBC lysis buffer. 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 were presented as the ratio to control.

8. Bioavailability and pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using Thermo Kinetica Software Version 5.0 (Thermo Fisher Scientific Inc., Miami, OK, USA). The parameter values were obtained by fitting to the pharmacokinetic model using the simplex algorithm. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) was calculated by a trapezoidal rule. The peak concentration (C_{max}) of nifedipine in plasma and time to reach C_{max} (T_{max}) were obtained by visual inspection of the data from the concentration–time curve. The terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. Total body clearance (CL/F) was calculated by dose/AUC. The absolute bioavailability (AB) was calculated by $AUC_{oral}/AUC_{i.v.} \times dose_{i.v.}/dose_{oral}$, and the relative bioavailability (RB) of nifedipine were calculated by $AUC_{nifedipine\ with\ licochalcone\ A}/AUC_{control}$. The metabolite–parent AUC ratio (MR) was calculated by $AUC_{dehydronifedipine}/AUC_{nifedipine}$.

9. Statistical analysis

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

C. Results

1. Inhibition of CYP3A4

The inhibitory effect of licochalcone A on CYP3A4 activity is shown in Figure 13. Licochalcone A inhibited CYP3A4 activity in a concentration-dependent manner. Licochalcone A inhibited CYP3A4 activity with an IC_{50} value of 3.9 μM.

2. Rhodamine-123 retention assay

The effect of licochalcone A on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cell is shown in figure 14. Accumulation of rhodamine-123, a P-gp substrate, was decreased in MCF-7/ADR cells overexpressing P-gp compared to that in MCF-7 cells lacking P-gp. The concurrent use of licochalcone A ($P < 0.05$ for 3 μM, $P < 0.01$ for 10 μM) enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner. This result suggests that licochalcone A significantly inhibited P-gp activity.

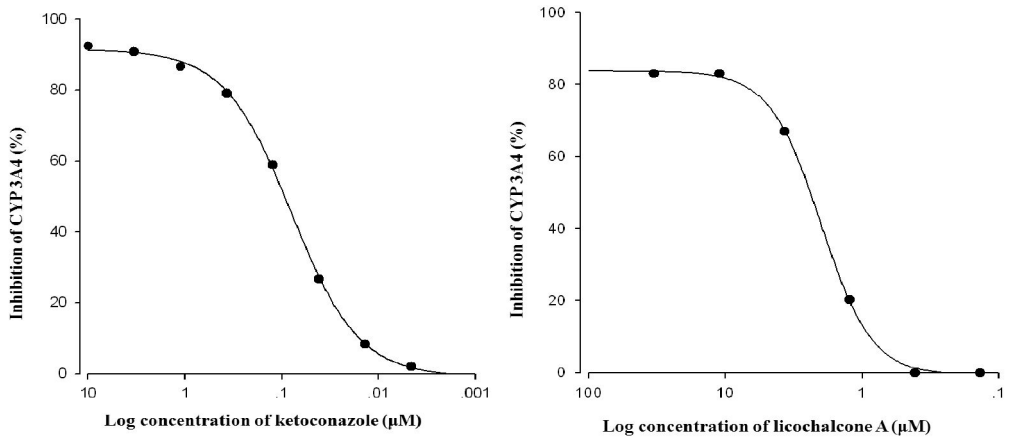

Figure 13. Inhibitory effect of ketoconazole and licochalcone A on CYP3A4 activity. The results were expressed as the percent of inhibition.

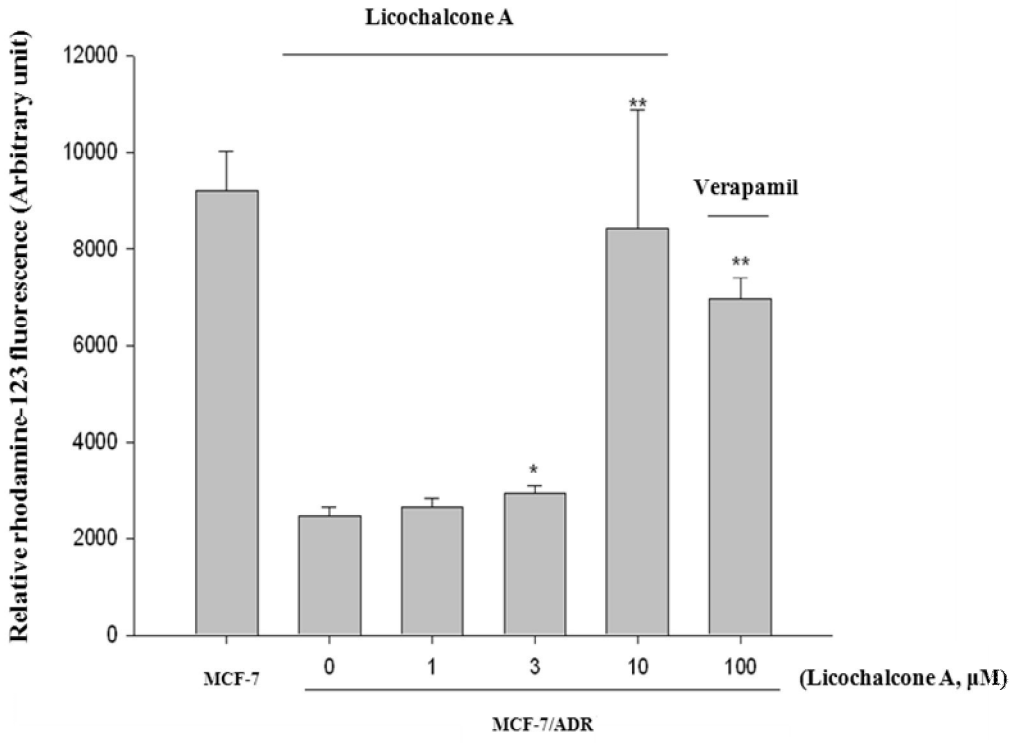

Figure 14. Effects of licochalcone A on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD (n = 6).

* $P < 0.05$, ** $P < 0.01$, significant difference compared to positive control (Verapamil).

3. Effect of licochalcone A on the bioavailability of oral nifedipine

The mean plasma concentration-time profiles of nifedipine in the presence and absence of licochalcone A (0.4, 2 and 10 mg/kg) are shown in Figure 15. The bioavailability and pharmacokinetic parameters of nifedipine are summarized in Table 4. Licochalcone A significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased the area under the plasma concentration-time curve from time zero to time infinity ($AUC_{0-\infty}$) of nifedipine by 35.3-55.7%. The peak concentration (C_{max}) of nifedipine were increased by 19.6-52.4%. The time to reach peak plasma concentration (T_{max}) were significantly shortened by licochalcone A . The total body clearance (CL/F) was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) decreased (26.0-35.6%) by licochalcone A. Accordingly, the absolute bioavailability (AB) values of nifedipine in the presence of licochalcone A were significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) higher (34.8-55.7%) than that of the control group. Licochalcone A increased the relative bioavailability (RB) of nifedipine by 1.35- to 1.56-fold. There were no significant differences in the terminal half-life ($t_{1/2}$) of nifedipine in the presence of licochalcone A.

4. Effect of licochalcone A on the bioavailability of dehydronifedipine

The plasma concentration-time profiles of dehydronifedipine are shown in Figure 16. The bioavailability and pharmacokinetic parameters of dehydronifedipine are summarized in Table 5. The Licochalcone A significantly (10 mg/kg, $P < 0.05$) increased the area under the plasma concentration-time curve from time zero to time infinity ($AUC_{0-\infty}$) of dehydronifedipine by 32.8%. The MR ratios were significantly ($P < 0.05$, 10 mg/kg) decreased (16.7%) by licochalcone A, suggesting that the formation of dehydronifedipine was considerably altered by licochalcone A. Thus, the increased bioavailability of nifedipine by licochalcone A may be mainly due both to the inhibition of P-gp activity in the small intestine and to the inhibition of CYP3A4 activity in the small intestine and/or in the liver by licochalcone A.

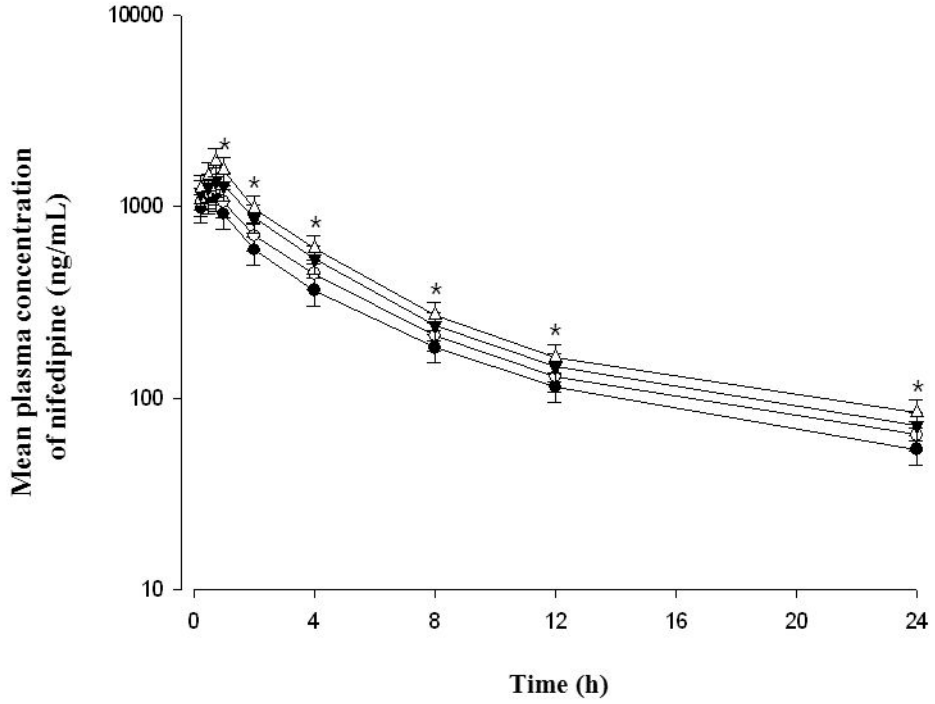

Figure 15. Mean plasma concentration-time profiles of nifedipine after oral administration of nifedipine (10 mg/kg) to rats in the presence and absence of licochalcone A (0.4, 2 or 10 mg/kg).

(mean ± SD, $n = 6$). (●) nifedipine alone, (○) with 0.4 mg/kg licochalcone A, (▼) with 2 mg/kg licochalcone A, (△) with 10 mg/kg licochalcone A.

* $P < 0.01$, significant difference compared to the control group given nifedipine alone.

Table 4. Mean bioavailability and pharmacokinetic parameters of nifedipine after oral administration of nifedipine (10 mg/kg) to rats in the presence or absence of licochalcone A

Parameters	Nifedipine (control)	Nifedipine + Licochalcone A		
		0.4 mg/kg	2 mg/kg	10 mg/kg
$AUC_{0-\infty}$ (ng h/ mL)	5930 ± 1067	6926 ± 1246	8025 ± 1364*	9234 ± 1569**
C_{max} (ng/ mL)	1130 ± 192	1222 ± 219	1352 ± 229*	1722 ± 297**
T_{max} (h)	0.71 ± 0.19	0.71 ± 0.19	0.63 ± 0.14	0.63 ± 0.14
CL/F (mL/hr/kg)	28.1 ± 5.1	24.1 ± 4.3	20.8 ± 3.5*	18.1 ± 3.1**
$t_{1/2}$ (h)	9.5 ± 1.8	9.7 ± 2.0	9.8 ± 2.0	10.1 ± 2.2
AB (%)	15.8 ± 2.7	18.4 ± 3.3	21.3 ± 3.6*	24.6 ± 4.2**
RB (%)	100	117	135	156

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

(mean ± SD, $n = 6$).

* $P < 0.05$, ** $P < 0.01$, significant difference compared to the control group given nifedipine alone.

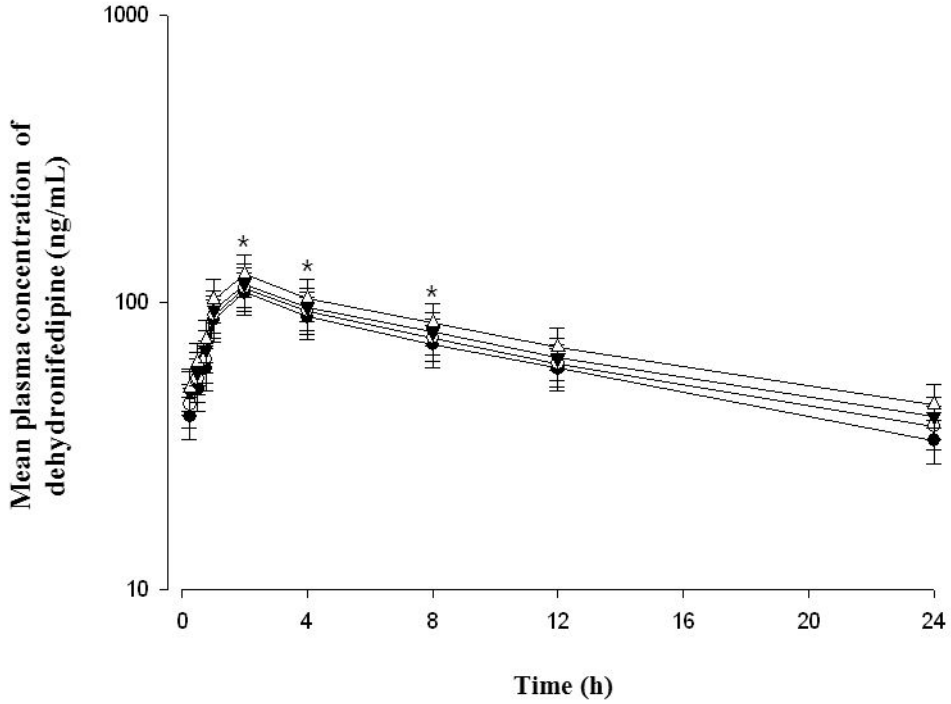

Figure 16**.** Mean plasma concentration-time profiles of dehydronifedipine after oral administration of nifedipine (10 mg/kg) to rats in the presence and absence of licochalcone A (0.4, 2 or 10 mg/kg).

(mean ± SD, *n* = 6). (●) nifedipine alone, (○) with 0.4 mg/kg licochalcone A, (▼) with 2 mg/kg licochalcone A, (△) with 10 mg/kg licochalcone A.

* $P < 0.05$, significant difference compared to the control group given nifedipine alone.

Table 5. Mean bioavailability and pharmacokinetic parameters of dehydronifedipine after oral administration of nifedipine (10 mg/kg) to rats in the presence or absence of licochalcone A

Parameters	Nifedipine (control)	Nifedipine + Licochalcone A		
		0.4 mg/kg	2 mg/kg	10 mg/kg
$AUC_{0-\infty}$ (ng h/ mL)	2144 ± 385	2387 ± 385	2580 ± 465	2847 ± 484*
C_{max} (ng/ mL)	108 ± 19	120 ± 19	123 ± 21	125 ± 23
T_{max} (h)	2.00 ± 1.09	2.00 ± 1.09	2.17 ± 0.98	2.17 ± 0.98
$t_{1/2}$ (h)	14.4 ± 2.5	15.7 ± 2.5	16.6 ± 3.0	16.9 ± 3.0
RB (%)	100	111	120	133
MR (%)	0.36 ± 0.06	0.34 ± 0.06	0.32 ± 0.05	0.30 ± 0.05*

$AUC_{0-\infty}$, 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; RB, relative bioavailability; MR, metabolite-parent ratio ($AUC_{dehydronifedipine}/AUC_{nifedipine}$).

(mean ± SD, $n = 6$).

* $P < 0.05$, significant difference compared to the control group given nifedipine alone.

5. Effect of licochalcone A on the bioavailability of intravenous nifedipine

Mean arterial plasma concentration-time profiles of nifedipine following an intravenous administration of nifedipine (2.5 mg/kg) to rats in the presence or absence of licochalcone A (2 and 10 mg/kg) are shown in Figure 17, while the corresponding bioavailability and pharmacokinetic parameters are shown in Table 6. The $AUC_{0-\infty}$ and CL_t of nifedipine were changed, but was not statistically significant compared to those in the control. The $t_{1/2}$ of nifedipine was also prolonged, but this increase was not statistically significant. The pharmacokinetics of intravenous nifedipine was not affected by the concurrent use of licochalcone A in contrast to those of oral nifedipine. Accordingly, the enhanced oral bioavailability in the presence of licochalcone A, while there was no significant change in the bioavailability and pharmacokinetics of intravenous nifedipine, may be mainly due to inhibition of the CYP3A-mediated metabolism of nifedipine in the small intestine and/or in the liver by licochalcone A rather than renal elimination of nifedipine.

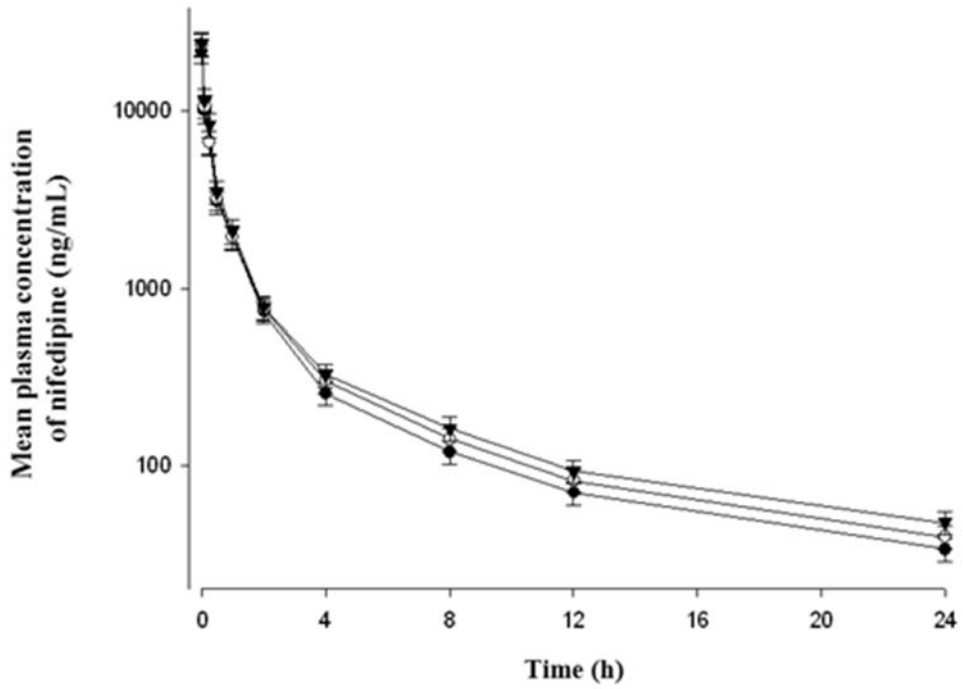

Figure 17. Mean plasma concentration-time profiles of nifedipine after intravenous administration of nifedipine (2.5 mg/kg) to rats in the presence and absence of licochalcone A (2 or 10 mg/kg). (mean ± SD, n = 6). (●) nifedipine alone, (○) with 2 mg/kg licochalcone A, (▼) with 10 mg/kg licochalcone A.

Table 6. Mean bioavailability and pharmacokinetic parameters of nifedipine after intravenous (2.5 mg/kg) administration to rats in the presence or absence of licochalcone A

Parameters	**Nifedipine (control)**	**Nifedipine + Licochalcone A**	
		2 mg/kg	10 mg/kg
$AUC_{0-\infty}$ (ng h/ mL)	9412 ± 1693	10108 ± 1718	11106 ± 1901
CL_t (mL/hr/kg)	9.2 ± 1.6	9.2 ± 1.6	9.0 ± 1.5
$t_{1/2}$ (h)	9.2 ± 1.7	9.3 ± 1.7	9.6 ± 1.9
RB (%)	100	107	118

$AUC_{0-\infty}$, total area under the plasma concentration–time curve from time zero to infinity; CL_t, total body clearance; $t_{1/2}$, terminal half-life; RB, relative bioavailability.

(mean ± SD, $n = 6$).

D. Discussion

With the great interest in herbal components as alternative medicines, much effort is currently being expended to identify natural compounds of plant origin that modulate P-gp and metabolic enzymes, however, there is far less information on the pharmacokinetic interactions between herbal components and medicines. More preclinical and clinical investigations on the herbal constituents-drug interaction should be performed to prevent potential adverse reactions or to utilize those interactions for a therapeutic benefit. Therefore, the present study evaluated the effect of licochalcone A, a naturally occurring flavonoid, on the pharmacokinetics of nifedipine in rats to examine a potential drug interaction between licochalcone A and nifedipine via the dual inhibition of CYP3A4 and P-gp.

P-gp is co-localized with CYP3A4 in the apical membrane of the intestine,[41,42] and they act synergistically in regulating the first-pass metabolism and bioavailability of many orally drugs. The inhibitory effect of licochalcone A against CYP3A4-mediated metabolism was confirmed by the employment of recombinant CYP3A4 enzyme. As shown in Figure 13, licochalcone A exhibited inhibitory effect against CYP3A4-mediated metabolism with IC_{50} of 3.9 μM. Furthermore, the cell-based assay using rhodamine-123 indicated that licochalcone A significantly (3 μM, $P < 0.05$; 10 μM, $P < 0.01$) inhibited P-gp-mediated drug efflux (Figure 14). Those results appeared to be consistent with the findings of previous studies.[43,44]

Those results suggest that licochalcone A might be effective to improve the bioavailability of nifedipine, a substrate of CYP3A4 and P-gp. Therefore, the bioavailability and pharmacokinetic characteristics of nifedipine were evaluated in the absence and the presence of licochalcone A in rats.

CYP3A4 expressed in rat is similar and corresponding to the action of CYP3A4 in human.[32,35-37,45]

As summarized in Table 4, licochalcone A significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased $AUC_{0-\infty}$ of nifedipine by 35.3-55.7%. The peak concentration (C_{max}) of

nifedipine were increased by 19.6-52.4%. The time to reach peak plasma concentration (T_{max}) were significantly shortened by licochalcone A . The total body clearance (CL/F) was significantly decreased (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) by licochalcone A. The absolute bioavailability (AB) of nifedipine in the presence of licochalcone A was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) higher (34.8-55.7%) than that in the control group. Licochalcone A inhibited CYP3A4 isozymes and P-gp activity in present study. These results appeared to be consistent with the finding that myricetin, a flavonoid, is a CYP3A4 and P-gp inhibitor and significantly increased the $AUC_{0-\infty}$ and C_{max} of losartan.[36] These results were also consistent with the findings of some previous studies,[32-35] in which epigallocatechin and hesperidin increased bioavailability of verapamil (a substrate of CYP3A4 and P-gp) in rats, and in which morin and hesperidin increased $AUC_{0-\infty}$ and C_{max} of diltiazem (a substrate of CYP3A4 and P-gp) in rats. Piao *et al.*[37] reported that morin, a flavonoid, significantly enhanced the bioavailability of nicardipine that nicardipine might be due to the inhibition of P-gp and intestinal metabolism by morin.

Also those results appeared to be consistent with the findings of some previous studies.[31,45] Zaidenstein *et al.*[31] reported significant differences in the pharmacokinetic parameters of losartan and its metabolite EXP-3174 as a result of concomitant intake of grapefruit juice, a CYP3A4 inhibitor.

As summarized in Table 5, licochalcone A significantly decreased metabolite-parent AUC ratios (MR). The MR, a characteristic of the magnitude of metabolic conversion, was significantly changed by the inhibition of CYP3A4-mediated metabolism of losartan after co-administration of grapefruit juice.[31] Epigallocatechin and morin significantly decreased MR of verapamil and diltiazem, respectively.[32,34] Those studies in conjunction with our present findings, suggest that the combination of nifedipine and CYP (CYP3A4) inhibitors could result in a significant pharmacokinetic drug interaction.

In the present study, therefore, the decrease in the metabolite-parent AUC ratios (MR) of nifedipine might be mainly due to the inhibitory effect of licochalcone A on the first-pass effects (metabolism) in the liver and/or in the small intestine. Licochalcone A did not

significantly change the pharmacokinetic parameters of intravenous administration of nifedipine (Table 6), suggesting that licochalcone A may improve the oral bioavailability of nifedipine by more increasing the absorption or reducing intestinal metabolism of nifedipine by the inhibition of CYP3A rather than renal elimination by licochalcone A.

Therefore, the enhanced bioavailability of nifedipine might be mainly due to inhibition of the CYP3A -mediated metabolism of nifedipine in the small intestine and/or in the liver and to inhibition of the P-gp efflux transporter in the small intestine by licochalcone A.

Although potential adverse effects, this interaction may provide a therapeutic benefit whereby it enhances bioavailability and lowers the dose administered. Since the present study raised the awareness about the potential drug interactions by concomitant use of licochalcone A, a natural flavonoid, with nifedipine, the clinical significance of this finding need to be further evaluated in the clinical studies.

Therefore, the dose of nifedipine should be adjusted when coadministered with licochalcone A for rational dosage regimen.

E. Conclusion

The increased bioavailability of nifedipine in the presence of licochalcone A, OTC medicine, might be due to an inhibition of the P-gp-mediated efflux transporter in the small intestine and to inhibition of CYP 3A-mediated metabolism in the small intestine and/or in the liver and to the reduction of the total body clearance rather than to a reduction of renal elimination by licochalcone A.

Concomitant use of nifedipine with licochalcone A, herbar medicine, may require close monitoring for potential drug interactions. However, the clinical importance of these findings should be further investigated in clinical trials.

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Abstract

The purpose of this study was to investigate the possible effects of licochalcone A on the bioavailability and pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, in rats. The bioavailability and pharmacokinetic parameters of nifedipine and dehydronifedipine were determined after oral and intravenous administration of nifedipine to rats in the presence and absence of licochalcone A (0.4, 2 and 10 mg/kg).

The effect of licochalcone A on P-glycoprotein (P-gp) and cytochrome P450 (CYP) 3A4 activity was evaluated. Licochalcone A inhibited CYP3A4 enzyme activity in a concentration-dependent manner with a 50% inhibition concentration (IC_{50}) of 3.9 μM. In addition, licochalcone A significantly enhanced the cellular accumulation of rhodamine-123 in MCF-7/ADR cells overexpressing P-gp.

The bioavailability and pharmacokinetic parameter of nifedipine were significantly changed by the presence of kaempferol compared with the control group. The areas under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak concentration (C_{max}) of nifedipine were significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased by 35.3-55.7% and 19.6-52.4%, respectively, with licochalcone A. The total body clearance (CL/F) was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) decreased by licochalcone A (26.0-35.6%). Consequently, the absolute bioavailability (AB) of nifedipine in the presence of licochalcone A was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) higher (34.8-55.7%) than that of the control group. Moreover, the relative bioavailability (RB) of nifedipine was 1.35- to 1.56-fold greater than that in the control group. The metabolite-parent AUC ratio (MR) in the presence of licochalcone A (10 mg/kg) significantly ($P < 0.05$) decreased compared to the control group.

This result implied that licochalcone A effectively inhibited the metabolism of nifedipine. The increased bioavailability of nifedipine in the presence of licochalcone A might be due to an inhibition of the P-gp-mediated efflux transporter in the small intestine and CYP3A-mediated metabolism in the small intestine and/or in the liver and/or to a reduction of total body

clearance rather than to a reduction of renal elimination of nifedipine by licochalcone A.

Key words: Nifedipine; dehydronifedipine; licochalcone A ; CYP3A4; P-gp; pharmacokinetics; bioavailability; rats

Abstract

Effects of kaempferol and licochalcone A on the bioavailability of nifedipine in rats

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The aim of this study was to investigate the possible effects of kaempferol and/or licochalcone A on the bioavailability and pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, in rats. The bioavailability and pharmacokinetic parameters of nifedipine and dehydronifedipine were determined after oral and intravenous administration of nifedipine to rats in the presence and absence of kaempferol and/or licochalcone A (0.4, 2 and 10 mg/kg). The effect of kaempferol and/or licochalcone A on P-glycoprotein (P-gp) and cytochrome P450 (CYP) 3A4 activity were evaluated. Kaempferol and licochalcone A inhibited CYP3A4 enzyme activity in a concentration-dependent manner with a 50% inhibition concentration (IC_{50}) of 9.8 μM and 3.9 μM. In addition, kaempferol and licochalcone A significantly enhanced the cellular accumulation of rhodamine-123 in MCF-7/ADR cells overexpressing P-gp. The areas under the plasma concentration-time curve ($AUC_{0-\infty}$) of nifedipine was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased by 29.3-45.4% and 35.3-55.7%, respectively, with kaempferol and/or licochalcone A . The peak concentration (C_{max}) of nifedipine were significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) increased by 41.8% and 19.6-52.4%, respectively, with kaempferol and/or licochalcone A. The total body clearance (CL/F) was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) decreased by 22.8-31.3% and 26.0-35.6%, respectively, with kaempferol and/or licochalcone A. Consequently, the absolute bioavailability (AB) of nifedipine in the presence of kaempferol and/or

licochalcone A was significantly (2 mg/kg, $P < 0.05$; 10 mg/kg, $P < 0.01$) higher by 28.5-44.9% and 34.8-55.7% than that of the control group. Moreover, the relative bioavailability (RB) of nifedipine was 1.29-1.45-fold and 1.35-1.56-fold greater, respectively, with kaempferol and/or licochalcone A than that in the control group. The metabolite-parent AUC ratio (MR) in the presence of kaempferol and/or licochalcone A (10 mg/kg) significantly ($P < 0.05$) decreased compared to the control group. This result implied that kaempferol and licochalcone A effectively inhibited the metabolism of nifedipine. The increased bioavailability of nifedipine in the presence of kaempferol and/or licochalcone A might be due to an inhibition of the P-gp-mediated efflux transporter in the small intestine and CYP3A-mediated metabolism in the small intestine and/or in the liver and/or to a reduction of total body clearance rather than to a reduction of renal elimination of nifedipine by kaempferol and/or licochalcone A. Therefore, the dose of nifedipine should be adjusted when coadministered with kaempferol and/or licochalcone A for rational dosage regimen.

Key words: Nifedipine; dehydronifedipine; kaempferol; licochalcone A; CYP3A4; P-gp; pharmacokinetics; bioavailability; rats