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Mesenteric Lymphatic Absorption and the Pharmacokinetics of Naringin and Naringenin in the Rat

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ABSTRACT: The hypothesis for this study was that flavanoids and their glycoside are absorbed mainly through the portal vein to enter the liver for biotransformation and are only partially absorbed through the lymphatic duct. To verify this hypothesis, an unconscious, mesenteric lymphatic/portal vein/jugular vein/bile duct/duodenum-cannulated rat model was developed. Naringin was administered at dosages of 600 and 1000 mg/kg, and naringenin was given at 100 and 300 mg/kg by intraduodenal administration. Blood samples collected from the portal vein and jugular vein as well as lymphatic fluid were prepared by protein precipitation and then analyzed by high-performance liquid chromatography with photodiode array detection (HPLC-DAD). Analyses of these samples were doubly confirmed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The results show that, after intraduodenal administration, both compounds were mainly absorbed into portal blood rather than mesenteric lymph, and most of the intact analyte would be eliminated through bile excretion. The area under the concentration (AUC) ratio was defined to represent the absorption ratio for portal vein [$AUC_{portal}/AUC_{(portal+lymph)}$] and lymph fluid [$AUC_{lymph}/AUC_{(portal+lymph)}$]. The results indicate that the portal and lymphatic absorptions for naringin were around 95 and 5.0%, respectively. The respective absorptions for naringenin were around 98 and 2%, respectively.

KEYWORDS: *first-pass effect, flavanoids, lymphatic absorption, pharmacokinetics, portal vein absorption*

INTRODUCTION

Portal and lymphatic absorption are the two major absorption pathways for orally administered substances, and administration is the most common route for the intake of foods, medicines, and other substances. After being ingested into the gastrointestinal tract, substances are digested by enzymes and absorbed through the intestinal cells, whence these molecules are transported into either intestinal capillaries or lacteal ducts. Most molecules transported via the portal vein to the liver are further metabolized or directly eliminated before entering the systemic circulation. This phenomenon is known as the hepatic first-pass effect, and it causes low bioavailability of many drugs.[1,2] On the other hand, some lipophilic or macromolecular substances may associate with lymph lipoproteins in the enterocyte and be transported to the systemic circulation via the intestinal lymphatic system. Thus, these substances may enter the general circulation without being metabolized by the liver, thereby increasing their bioavailability.[1,3]

Thus, the absorption route can significantly affect the fate of ingested substances. Studies dealing with how drugs are absorbed and transported into the body via the portal vein and the intestinal lymphatic system reveal that the diffusion of drugs across the blood capillary endothelium is limited by many factors, such as molecular size, solubility, and other properties of the substances.[4] Macromolecular, colloidal, or lipophilic materials have access to the intestinal lymphatics more easily than to the portal vein. Furthermore, lymphatic transport has been shown to be a major absorption route for some highly lipophilic drugs and xenobiotics following oral administration, such as halofantrine,[3,5,6] cyclosporine,[7] testosterone derivatives,[8,9] mepitiostane,[10−13] lipid-soluble vitamins and their derivatives,[14] cholorophenothane (DDT) and its analogues,[15,16] organochlorine compounds,[2] and still others.[17] This phenomenon demonstrates that in the gastrointestinal tract, macromolecular or lipophilic substances are absorbed mainly through lymphatics, rather than blood capillaries.

Flavanoids are a group of polyphenolic compounds with health-related properties that are widely distributed in vegetables, fruits, cocoa, teas, wines, and so on. Several studies have shown that the flavanoids in grapefruit juice, including quercetin and naringin, elevate the blood levels of some orally administered drugs by inhibiting the P-glycoprotein and cytochrome P450-mediated first-pass effects.[18] According to Murota and Terao,[19] quercetin can be transported by both intestinal capillaries and lacteal ducts. Our previous work also showed that quercetin could be absorbed into the mesenteric lymphatic duct. However, we found that rutin, the glycoside of quercetin, was more likely to be absorbed into intestinal capillaries than into lacteals.[20]

Naringin is the predominant flavanoid in grapefruit, and it has characteristics similar to those of rutin. The pharmacoki-

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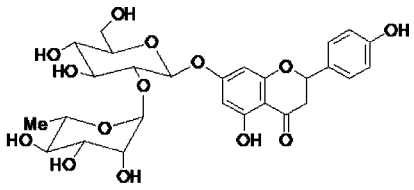
netics of naringin in the systemic circulation was reported by Tsai in 2002.[21] According to the findings, naringin was concentrated in the liver and bile by the processes of active transport, and the hepatobiliary excretion of naringin may not be related to the P-glycoprotein.[21] However, until now there have been no references discussing the partition of naringin or naringenin between the lymphatic and portal vein absorption after oral administration.

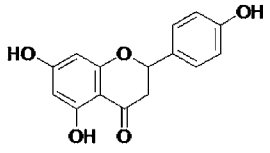
On the basis of pharmacokinetic concern, the flavanoids and their glycoside may process hepatobiliary excretion and enterohepatic circulation. Our hypothesis is that the flavanoids and their glycosides are absorbed mainly through the portal vein to enter the liver for biotransformation and are only partially absorbed through the lymphatic duct. To verify this hypothesis, we investigated the concentration of naringin or naringenin in the portal vein and mesenteric lymph in rats after intraduodenal administration. Simultaneous measurement of both portal vein and mesenteric lymph partitions can provide direct evidence for the different absorbabilities and pharmacokinetic profiles in each absorption system. This study develops a methodology using the cannulated ileocolic vein, common bile duct, mesenteric lymphatic duct, jugular vein, and duodenum for biological fluid sampling and drug administration in rats. This model allowed period sampling of mesenteric lymph fluid, portal blood, and jugular blood from cannulated rats. Naringin and naringenin were chosen to investigate the absorption pathway between the lymphatic and portal systems. HPLC-MS/MS was used to confirm the identification of the analytes in various biological fluids.

MATERIALS AND METHODS

Chemicals. Naringin and naringenin (Figure 1) were purchased from Sigma (St. Louis, MO, USA). Methanol and acetonitrile of



(A) naringin



(B) naringenin

Figure 1. Chemical structures of (A) naringin and (B) naringenin.

HPLC grade were obtained from E. Merck (Darmstadt, Germany). Triply deionized water (Millipore, Bedford, MA) was used in all of the experiments presented.

Animals. Specific pathogen-free, 8-week-old male Sprague–Dawley rats weighing around 300 g obtained from the Laboratory Animal Center of the National Yang-Ming University were used in the experiment. All animals were housed in temperature-controlled quarters (24 ± 1 °C) with a 12 h/12 h light/dark cycle. Food (Laboratory Rodent Diet 5001, PMI Feeds Inc., Richmond, IN, USA) and water were available ad libitum. Animal protocols were reviewed and approved by the Institutional Animal Experimentation Committee of National Yang-Ming University.

Surgical Procedures. For each experimental group, six male Sprague–Dawley rats were randomly chosen and had food withdrawn for 12 h before surgery. To facilitate viewing of the mesenteric lymph duct, sesame oil (1 mL per rat) was fed to the rats 30 min prior to anesthesia. The rats were anesthetized by an anesthetic (1 mL/kg, ip) composed of urethane (1 g/mL) and α-chloralose (0.1 g/mL) at the beginning of all experiments and remained anesthetized throughout the experimental period. During the experiment, the body temperature of the experimental rats was maintained with a heating pad set at 37 °C. Several papers have described that the absorption and metabolism of anesthetized animals are both lower than those of conscious animals. These interactions appeared to be differential and variable, depending not only on the anesthetic regimens but also on the pharmacokinetic behaviors of the coadministered compounds.[22] Considering the difficulty of multiple sampling, the anesthetized rat was finally selected as an experimental model.

The right jugular vein was implanted with polyethylene tubing for systemic circulation blood sampling. The mesenteric lymph duct cannulation method was as described by Warshaw[23] and modified by Chen et al.[20] (Figure 2). Briefly, the right side of the abdominal flank

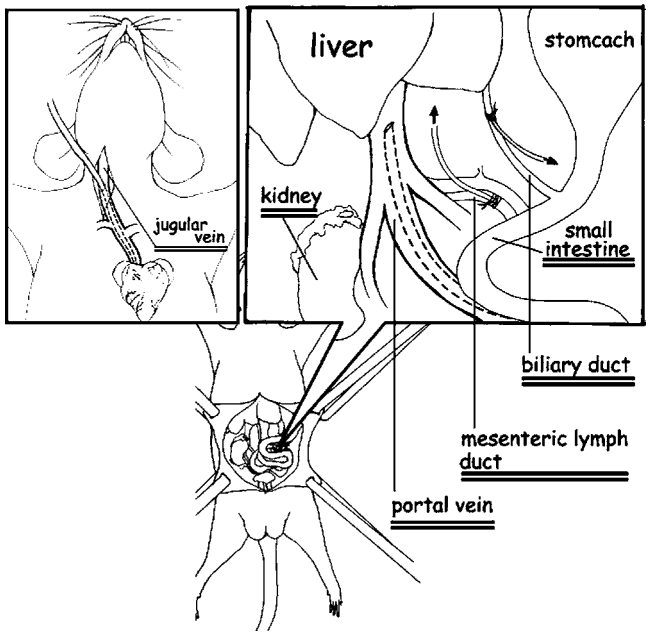


Figure 2. Schematic illustration of catheterization of the jugular vein, portal vein, and mesenteric lymphatic duct in the rat. Naringin and naringenin were individually administered via PE50 cannula at the duodenum.

was clipped and disinfected before surgery; an incision was made in the right abdomen to expose the mesenteric lymph duct, ileocolic vein, bile duct, and duodenum. The mesenteric lymph duct was milky-white after sesame oil administration prior to surgery and was cannulated with heparinized PE-10 tubing (0.28 mm i.d., 0.61 mm o.d.) of 10 cm in length. Cyanoacrylate glue was used for fixing the cannula in place.

Next, normal saline rinsed cotton swabs were used to pull out the cecum from the abdomen, and the ileocolic vein cannulation method described by Nishihira et al.[24] was used with a slight modification as the cannulation protocol. The cecal vein was cannulated carefully with heparinized silicone tubing (0.64 mm i.d., 1.20 mm o.d.) 15 cm in length, and the catheter was further pushed toward the portal vein until it reached the major portal vein. After insertion, the catheter was ligated and a drop of cyanoacrylate glue was used to fix the cannula in place and prevent bleeding.

The bile duct was cannulated with heparinized PE-10 tubing and drainage back to the duodenum as the cannulation method adapted and modified from Tsai et al.[25−27] PE-50 tubing (0.58 mm i.d., 0.965 mm o.d.) of 10 cm in length was introduced about 1 cm down the

Table 1. Experimental Parameters of Analytes for MRM Detection Mode in the LC-MS/MS

compound	precursor ion (amu)	product ion (amu)	DP[a] (V)	FP[a] (V)	EP[a] (V)	CE[a] (eV)	CXP[a] (V)	retention time (min)
naringin	581.2	273.2	90	300	11	20	24	3.25
naringenin	273.2	153.2	55	215	12	27	25	5.40
hesperidin	611.2	303.2	50	250	10	30	21	3.30

[a]DP, declustering potential; FP, focusing potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential.

duodenum through the fundus of the stomach. Because of its instability in the acidic stomach milieu, naringin was administered intraduodenally to avoid naringin being degraded to its aglycone form in the stomach. However, the intestinal environment, such as osmotic pressure, pH value, intestinal peristalsis, etc., still affects the pharmacokinetics of the analytes. After all cannulation was complete, the fundus incision was closed by suture, and the animals were allowed to recover in a quiet condition for 30 min and kept warm on the heating pad set to 37 °C for the duration of sampling.

Mesenteric Lymphatic and Portal Vein Absorption of Naringin and Naringenin. *Drug Administration and Sampling.* Both naringin and naringenin were suspended in carboxymethyl cellulose (2% in water, w/v) solution, and the concentrations of both drugs were 600 and 100 mg/mL, respectively. The doses of naringin and naringenin were adjusted by volume to achieve experimental dosages administered to the rat at 600 and 1000 mg/kg for naringin and 100 and 300 mg/kg for naringenin via the duodenal cannula with an injection volume of 1 mL/kg. According to the results from Nogata et al.,[28] the concentration of naringin in grapefruit weighs about 1360 mg/100 g when fresh. After conversion into the present study, the naringin dosages of 600 and 1000 mg/kg were approximately 44.11 and 73.53 g/kg of fresh grapefruit weight, respectively. However, due to the low uptake rate, the dose of test molecules should be increased to measure them in mesenteric lymph. The lymph and bile samples were gathered via the mesenteric lymph duct and bile duct cannula into heparin-rinsed Eppendorf, respectively, and collected every 30 min at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min after drug administration. Plasma samples were collected separately from the jugular vein and portal vein, at the time points of 0, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min after drug administration. To minimize degradation, all samples were stored at approximately −70 °C until bioanalysis.

Sample Pretreatment for HPLC-PDA Analysis. Aliquots of 50 μL samples of jugular plasma, portal plasma, or mesenteric lymph fluid were transferred to a clean Eppendorf. An aliquot of 100 μL of internal standard solution (rutin 100 μg/mL in acetonitrile) was added into the Eppendorf and vortexed for 1 min. After mixing, the sample was centrifugated at 16110*g* for 10 min at 4 °C. Then the upper organic layer was transferred into a new Eppendorf and dried at room temperature. The dried residue was reconstituted in dilute solution (acetonitrile/10 mM ammonium acetate = 80:20, v/v) before HPLC-PDA analysis.

Liquid Chromatography. Liquid chromatography analysis was performed on a Shimadzu (Kyoto, Japan) system consisting of an LC-20AT pump, an SIL-20AC autosampler, and an SPD-M20A photodiode array detector. Separation was achieved in an analytical column (SB-Phenyl, 150 mm × 4.6 mm i.d., particle size = 5 μm, Agilent, Santa Clara, CA, USA).

The mobile phases for chromatographic delivery were 10 mM ammonium acetate in water, adjusted to pH 3.75 by acetic acid (solvent A), and acetonitrile (solvent B); both solutions were filtered (Millipore 0.45 μm) and degassed prior to use. Output signals from the HPLC-PDA were integrated via a Class-VP 7.0 Client/Server Chromatography Data System (Shimadzu). The mobile phase gradient program applied was 80:20 (A/B) at 0 min, 60:40 (A/B) at 10 min, held for 2 min; 80:20 (A/B) at 15 min, held 5 min. The flow rate of the mobile phase was 1 mL/min. All samples were placed in a temperature-controlled autosampler at 15 °C before analysis. The optimal detection UV wavelength was set at 280 nm, and the sample injection volume was 20 μL.

Method Validation. For calibration standards, a stock solution containing naringin and naringenin (1 mg/mL) was prepared in methanol and stored at −20 °C. For the daily preparation of a standard mixture, portions of these stock solutions were thawed at 4 °C and diluted to the appropriate concentration with dilute solution. Calibration curves of naringin and naringenin were quantified in six individual runs, all of which were required to have a coefficient of determination (r^2) value of at least 0.995. The intra- and interday variabilities for all analytes were assayed at concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, and 10 μg/mL on the same day and on six consecutive days, respectively. The limit of quantification (LOQ) was defined as the lowest concentration of the linear range, and the limit of detection (LOD) was defined as the concentration of analyte spiked in blank matrix sample yielding a signal-to-noise ratio (S/N) of 3.

The accuracy (% bias) was calculated by the following equation: bias (%) = $[(C_{obs} - C_{nom})/C_{obs}] \times 100$, where C_{nom} is the nominal concentration and C_{obs} is the mean value of observed concentration. The relative standard deviation (RSD) was calculated from the observed concentrations by the equation RSD (%) = [standard deviation (SD)/mean of C_{obs}] × 100. Accuracy and precision values were defined as within ±15%.[29]

Recovery. The recoveries of naringin and naringenin from rat plasma and lymph fluid were assessed at three concentrations: 0.1, 1.0, and 10 μg/mL. Recovery (%) was calculated as the following formula: recovery = (peak area of analyte in biological fluid/peak area of analyte in dilute solution) × 100.

LC-MS/MS Analysis. *Instrumentation.* The LC-MS/MS system that was used consisted of an Applied Biosystems MDS Sciex API3000 triple-quadrapole mass spectrometer (Thornhill, ON, Canada) coupled to an Agilent 1100 series HPLC system (Palo Alto, CA, USA). The HPLC system was equipped with an LC binary pump, a microvacuum degasser, an autosampler thermostat, and an autosampler.

HPLC-MS/MS Conditions. Naringin and naringenin were separated on a C18 Gemini (150 mm × 2.0 mm, particle size = 5 μm, Phenomenex, Torrance, CA, USA) column. Column temperature was maintained at room temperature. The mobile phase composition was a mixture of 0.1% formic acid/methanol (30:70, v/v), which was filtered through a 0.22 μm nylon filter before use. Isocratic elution was used for separating analytes. The flow rate was 0.3 mL/min, and the total run time was 8.0 min for each injection. The injection volume was 20 μL, and the autosampler temperature was set at 15 °C. The injection solvent was 0.1% formic acid/methanol (30:70, v/v).

A Sciex API 3000 mass spectrometer was equipped with a turbo ion spray interface and used electrospray ionization (ESI) with an ion spray voltage of 4500 V. The turbo ion spray probe temperature was maintained at 350 °C. Nitrogen was used for curtain gas, nebulizer gas, and collision gas. The curtain gas flow, nebulizer gas flow, and collision gas flow were set at 10, 6, and 7 L/min, respectively. Data acquisition and processing were performed by the Analyst 1.4.1 software package (Sciex).

Precursor and product ions of these compounds were obtained by using a syringe pump to infuse the standard solutions into the API 3000 mass spectrometer. The flow rate of the syringe pump was 10 μL/min. For precursor and product ion scans, we selected m/z 581.2 → 273.2 for naringin, 273.2 → 153.2 for naringenin, and 611.2 → 303.3 for the internal standard, hesperidin, used for mass condition in the qualitative analysis. We applied 0.1 μg/mL standard solutions to optimize the mass spectrometer detection conditions in the presence of the LC mobile phase. The optimal parameters are shown in Table 1.

Positive ion multiple reaction monitoring (MRM) mode was used for mass spectrometer detection in the study.

Pharmacokinetics. The results are expressed as the standard error of the mean (SEM). Pharmacokinetic calculations were carried out using a noncompartmental model with the software WinNonlin Standard Edition, version 1.1 (Scientific Consulting Inc., Apex, NC, USA). The areas under a plot of drug concentration versus time curves (AUC) were calculated according to the trapezoidal method. The absorption ratio of portal vein and mesenteric lymph duct were estimated as follows: for portal blood = $AUC_{portal}/AUC_{(portal+lymph)}$; for mesenteric lymph fluid = $AUC_{lymph}/AUC_{(portal+lymph)}$.[2] The clearances of the drug (Cl/F) are considered as follows: Cl = dose/AUC. The time required to reduce the drug concentration by half is shown as half-life ($t_{1/2}$) and is expressed as $t_{1/2} = 0.693/K$, where K is the first-order rate constant. The volume of distribution (Vd/F) is evaluated as Vd = dose/C_0, where C_0 is the initial plasma concentration. The mean residence time (MRT) is estimated as MRT = AUMC/AUC, where AUMC is the area under the first moment curve. All data are presented as mean ± standard error mean.

Statistics. To compare the differences of pharmacokinetic parameters between naringin and naringenin in rat portal vein plasma and mesenteric lymph, Student's t test for paired observations was performed, and a P value of <0.05 was considered to be significant. All data are shown as the mean ± SEM.

RESULTS

Validation of HPLC-PDA System. The present validated HPLC-PDA system was employed to determine naringin and naringenin from rat jugular plasma, portal plasma, and mesenteric lymph fluid following intraduodenally administered naringin and naringenin. The calibration curves of obtained naringin and naringenin in biological samples were observed prior to HPLC-PDA analysis over the concentration range of 0.05−10 μg/mL with six individual analytical runs and normalized to the response of the internal standard, rutin. This was linearly related to the peak areas for both analytes in the chromatogram ($r^2 > 0.995$). The limit of detection (LOD) was defined as the concentration of analyte spiked in blank matrix sample yielding a signal-to-noise ratio (S/N) of 3, and the limit of quantification (LOQ) as that yielding a S/N of 10. The LOD and the LOQ values for both analytes were 0.01 and 0.05 μg/mL, respectively. There were no interfering endogenous peaks observed during the retention times of naringin, naringenin, and internal standard, eluted in the chromatograms of blank jugular plasma, portal plasma, or mesenteric lymph fluid.

The average recoveries ($n = 6$) of naringin and naringenin at three different concentrations (0.1, 1, and 10 μg/mL) and of the internal standard, rutin, at one concentration (10 μg/mL) were similar in jugular plasma, portal plasma, mesenteric lymph fluid, and bile. The recoveries of naringin in jugular plasma, portal plasma, and mesenteric lymph fluid were 100.1 ± 2.8, 99.7 ± 0.3, and 103.1 ± 1.6%, respectively. The recoveries of naringenin in jugular plasma, portal plasma, and mesenteric lymph fluid were 94.5 ± 1.6, 95.3 ± 1.0, and 95.1 ± 4.1%, respectively. The recoveries of rutin, internal standard in the HPLC-PDA system, in jugular plasma, portal plasma, mesenteric lymph fluid, and bile were 99.44 ± 1.03, 103.65 ± 1.04, 96.52 ± 0.99, and 110.65 ± 1.08%, respectively. The actual concentrations of naringin and naringenin in the rat jugular plasma, portal plasma, mesenteric lymph fluid, and bile were corrected by the respective recoveries.

Analyte Confirmation by LC-MS/MS. LC-MS/MS was used to identify the analytes of naringin, naringenin, and the internal standard hesperidin. Under ESI conditions, naringin gave $[M + H]^+$ at m/z 581.2, naringenin gave at $[M + H]^+$ at m/z 273.2, and the internal standard, hesperidin, gave $[M + H]^+$ at m/z 611.2. The precursor/product ion pairs at m/z 581.2 → 273.2, 273.2 → 153.2, and 611.2 → 303.3 were selected in the MRM mode for analysis of naringin, naringenin, and hesperidin, respectively.

Pharmacokinetics of Naringin in Biological Fluids. The naringin concentration versus time curves in jugular vein and portal vein following intraduodenal administration of 600 and 1000 mg/kg are illustrated in Figure 3A, and those in the mesenteric lymph fluid and bile are shown in Figure 3B. The pharmacokinetics of naringin in the above biological fluids are summarized in Table 2. After naringin was administered via duodenal cannula (600 and 1000 mg/kg), the average C_{max} of naringin in portal plasma occurred at 18.8 ± 3.8 min (determined the concentration reach t_{max} in portal plasma) and was significantly higher than that in mesenteric lymph fluid. The C_{max} of naringin was about 1.7-fold higher in bile than in jugular plasma. The area under the curve (AUC) of naringin in

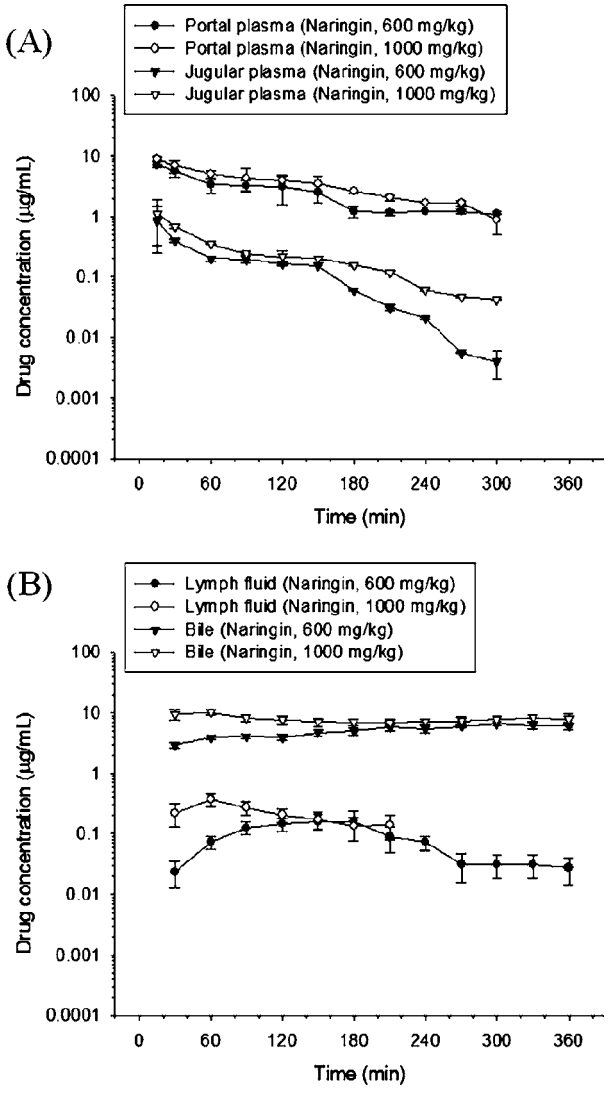


Figure 3. Concentration−time profiles of naringin in rat jugular plasma, portal plasma (A), and mesenteric lymph fluid (B) at naringin dosages of 600 and 1000 mg/kg intraduodenally. Data are presented as the mean ± standard error mean ($n = 6$).

Table 2. Pharmacokinetic Data of Naringin (600 and 1000 mg/kg) in Rat Jugular Plasma, Portal Plasma, and Lymph Fluid

	naringin (600 mg/kg, intraduodenally)				naringin (1000 mg/kg, intraduodenally)			
	portal plasma	lymph fluid	jugular plasma	bile	portal plasma	lymph fluid	jugular plasma	bile
AUC (min μg/mL)	745 ± 44.7	33.1 ± 4.3	46.8 ± 6.5	783 ± 14.6	1170 ± 7.9	74.1 ± 2.1	78.6 ± 9.6	1350 ± 44.0
t_{max} (min)	18.8 ± 3.8	186 ± 10.1	22.5 ± 4.3	280 ± 6.5	18.8 ± 3.8	56.7 ± 9.3	22.5 ± 5.6.	133 ± 5.5
C_{max} (μg/mL)	7.31 ± 0.04	0.21 ± 0.03	0.92 ± 0.04	6.92 ± 0.05	9.39 ± 0.35	0.42 ± 0.05	1.26 ± 0.48	9.32 ± 0.22
$t_{1/2}$ (min)	65.9 ± 4.2	77.8 ± 1.7	41.7 ± 3.5	256 ± 44.5	100 ± 11.1	113 ± 3.4	71.3 ± 5.8	713 ± 19.1
V_d/F (mL/kg)	77.9 ± 8.4	1880 ± 89.8	835 ± 9.5	138 ± 19.1	124 ± 14.2	1690 ± 18.8	12200 ± 408	499 ± 37.1
Cl/F (mL/min/kg)	0.8 ± 0.0	57.1 ± 19.5	13.5 ± 1.6	0.3 ± 0.1	0.9 ± 0.0	21.5 ± 4.6	133 ± 14.3	0.7 ± 0.2
MRT (min)	105.1 ± 0.5	200 ± 12.7	76.9 ± 7.8	370 ± 57.8	150 ± 16.3	197 ± 50.7	106 ± 10.1	679 ± 25.7
absorption ratio[a] (%)	95.9 ± 0.3	4.1 ± 0.2			93.9 ± 1.0	6.0 ± 0.0		

[a]Absorption ratio for portal vein = $AUC_{portal}/AUC_{(portal+lymph)}$; absorption ratio for lymphatic fluid = $AUC_{lymph}/AUC_{(portal+lymph)}$.

portal plasma was about 1.4-fold larger than in lymph fluid, and the AUC in bile was about 1.7-fold larger than in jugular plasma. After calculation, the absorption ratios of naringin in portal plasma and lymph fluid were about 95.9 and 4.1, respectively.

The pharmacokinetic data showed that AUC and C_{max} of naringin in portal plasma and bile were significantly higher than naringin in mesenteric lymph fluid and jugular plasma after intraduodenal administration (Table 2). These results suggest that naringin was mostly absorbed by the portal vein before entering the liver for biotransformation.

Pharmacokinetics of Naringenin in Biological Fluids. Figure 4 shows the concentration versus time curves and pharmacokinetic data of naringenin in jugular plasma, portal plasma (Figure 4A), mesenteric lymph fluid, and bile (Figure 4B), respectively. After naringenin administration (100 and 300 mg/kg), naringenin C_{max} in portal plasma occurred at about 15−26 min (t_{max} in portal plasma), which was sooner than the C_{max} of naringenin occurred in mesenteric lymph fluid. The C_{max} of naringenin in bile was about 1.2−2.6-fold higher than in jugular plasma. The AUC of naringenin in portal plasma was about 37−55-fold higher than that in lymph fluid, and the AUC was about 9.3-fold larger in bile than in jugular plasma. After calculation, the absorption ratios of naringenin in portal plasma and lymph fluid were about 97.9 and 2.2%, respectively (Table 3).

The pharmacokinetic data showed that naringenin in portal plasma had significantly larger AUC, shorter t_{max}, higher C_{max}, shorter $t_{1/2}$, smaller volume of distribution, and smaller clearance than naringenin in lymph fluid after naringenin administration (100 and 300 mg/kg, intraduodenally). On the other hand, the pharmacokinetic data also showed that naringenin in bile had significantly larger AUC, higher C_{max}, longer $t_{1/2}$, smaller clearance, and longer MRT than naringenin in jugular plasma.

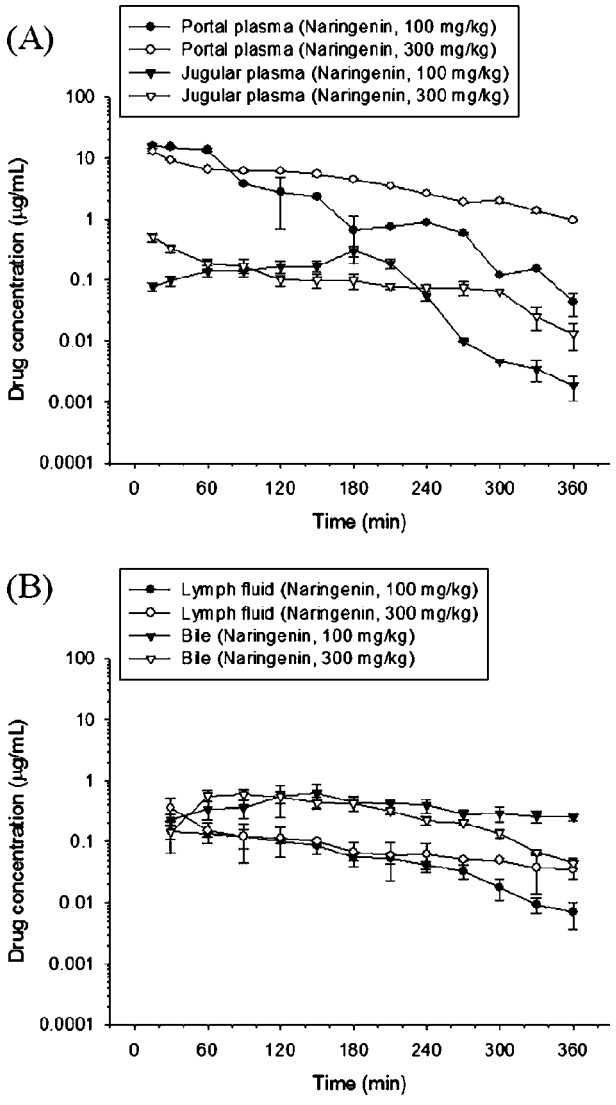


Figure 4. Concentration−time profiles of naringenin in rat jugular plasma, portal plasma (A), and mesenteric lymph fluid (B) at naringenin dosages of 100 and 300 mg/kg intraduodenally. Data are presented as the mean ± standard error mean (n = 6).

DISCUSSION

In our previous study,[20] an unconscious, mesenteric lymphatic/duodenum rat model was established and validated for the investigation of mesenteric lymphatic absorption with intraduodenally administered drugs. In the present study, we further modified and improved the mesenteric lymphatic/duodenum experimental model to a new unconscious rat model including mesenteric lymphatic duct, ileocolic vein, common bile duct, jugular vein, and duodenum cannulation for the sampling of various biological fluids and drug administration in the same experimental rat. By this experimental model, not only can mesenteric lymph fluid be collected to investigate the direct lymphatic absorption from the intestine but in addition the drug concentration can be simultaneously monitored via the hepatic portal system before the first-pass effect in the liver. This enables further understanding of the partition of an intraduodenally administered drug into the mesenteric lymph and portal vein after intestinal absorption.

Table 3. Pharmacokinetic Data of Naringenin (100 and 300 mg/kg) in Rat Jugular Plasma, Portal Plasma, and Lymph Fluid

	naringenin (100 mg/kg, intraduodenally)				naringenin (300 mg/kg, intraduodenally)			
	portal plasma	lymph fluid	jugular plasma	bile	portal plasma	lymph fluid	jugular plasma	bile
AUC (min μg/mL)	1340 ± 65.2	24.1 ± 2.9	39.2 ± 4.9	367.9 ± 11.7	1750 ± 6.9	46.5 ± 1.1	45.9 ± 2.4	426 ± 17.4
t_{max} (min)	26.3 ± 1.1	60.0 ± 1.3	180 ± 0.0	157 ± 3.9	15.0 ± 0.0	60.0 ± 1.5	15.0 ± 0.0	86.7 ± 7.8
C_{max} (μg/mL)	15.87 ± 0.79	0.18 ± 0.02	0.30 ± 0.04	0.78 ± 0.20	12.9 ± 0.86	0.51 ± 0.11	0.51 ± 0.06	0.66 ± 0.11
$t_{1/2}$ (min)	43.3 ± 1.9	46.2 ± 9.6	63.2 ± 4.6	844 ± 2.7	105 ± 2.6	116 ± 2.3	89.4 ± 10.5	263 ± 5.2
V_d/F (mL/kg)	4.7 ± 0.4	294 ± 6.8	251 ± 5.2	239 ± 4.3	25.9 ± 0.5	942 ± 9.7	837 ± 6.4	942 ± 6.9
Cl/F (mL/min/kg)	0.1 ± 0.0	4.4 ± 0.7	2.7 ± 0.4	0.3 ± 0.1	0.2 ± 0.0	8.1 ± 0.9	6.6 ± 0.3	2.7 ± 0.7
MRT (min)	68.0 ± 1.4	131 ± 5.5	148 ± 1.9	901 ± 3.6	155 ± 2.9	146 ± 2.4	127 ± 1.4	313 ± 4.9
absorption ratio[a] (%)	98.1 ± 0.1	1.9 ± 0.1			97.6 ± 0.3	2.4 ± 0.3		

[a]Absorption ratio for portal vein = $AUC_{portal}/AUC_{(portal+lymph)}$; absorption ratio for lymphatic fluid = $AUC_{lymph}/AUC_{(portal+lymph)}$.

After all body fluid has converged into systemic circulation, the jugular plasma can express the total absorption of substances after intraduodenal administration, and the bile collection from bile duct indicates the bile excretion of substances after hepatic metabolism. In other words, this mesenteric lymphatic/portal vein/jugular vein/bile duct/duodenum-cannulated rat model can be used to investigate the lymphatic and portal vein absorption, the uptake of drugs into systemic circulation, and bile excretion after intraduodenal administration. Thus, this experimental model can also be useful for the pharmacokinetic study of substances in the overall intestinal absorption, distribution, and elimination. To minimize the physiological effects of the vehicle and mimic the usual oral condition, carboxymethyl cellulose (2% in water, v/w) solution was chosen to suspend both naringin and naringenin.

The intestinal lymphatic absorption system has been shown to transport to the systemic circulation dietary fat, lipophilic substances, and xenobiotics such as organochlorine compounds,[2] halofantrine,[3,5,6] cyclosporine,[7] testosterone derivatives,[8,9] mepitiostane,[10−13] lipid-soluble vitamins and their derivatives, [14] chlorophenotane (DDT) and its analogues,[15,16] and other lipophilic drugs.[17] However, Porter[30] indicated that the fluid flow rate of portal blood is approximately 500-fold more than intestinal lymphatics, and the lipid content of the lymph is only of the order of 1−2% during peak lipid transport. This means that between intestinal lymph and portal blood the effective mass ratio is on the order of 1:50000, so small molecules and water-soluble drugs will have very low absorption by mesenteric lymphatic transport. On the other hand, lipid solubility is another important factor to consider in the partition coefficient for portal/mesenteric lymphatic absorption.[30]

After intestinal absorption and hepatic metabolism, the pharmacokinetic data of naringin and naringenin in jugular plasma and bile show that these two drugs in bile had significantly larger AUC and higher C_{max}, longer $t_{1/2}$, smaller clearance, and longer MRT than both in jugular plasma, and this result indicates that most of the absorbed naringin and naringenin were eliminated through bile excretion, with only a little being able to pass through the first-pass effect and enter systemic circulation. Therefore, the high percentage of bile excretion could be the major factor for the low bioavailability and lower recovery of naringin and naringenin in systemic circulation after oral administration.

On the basis of the multiple sampling method described above, our pharmacokinetic data demonstrate that the trend of AUC for naringin in bile and portal vein is much higher than those in jugular vein and lymph fluid, which suggests that the portal vein is the major absorption route and that hepatobiliary excretion takes place in naringin (Table 2). However, the portal vein AUC of the aglycone naringenin is 4-fold greater than that in bile, which suggests that the portal vein is still the main route for naringenin absorption but the amount of hepatobiliary excretion is much less than that of glycoside naringin (Table 3). The biotransformation commonly occurs in the intestinal hepatic system, and our experimental data clearly demonstrate that the enterohepatic circulation of naringin is higher than that of in naringenin.

The conventional pharmacokinetic model for intestinal absorption and lymphatic transportation has been reported by in vitro and in vivo studies. The Caco-2 cell experimental method was commonly used in in vitro experiments to assess the hydrophilic and lipophilic drugs' potential pathways in lymphatic transport.[31] In vitro experiment mimics the transit times and pH conditions in the intestinal environment and provides essential information on the mechanism of drug absorption but may not predict or correlate with in vivo performance. The concentration measurement in the lymph duct has been confirmed by estimating the total amount of drug lymphatic transportation for the drug, but a skillful surgical technique is required.[32,33]

Compared with the above experimental methods, simultaneous measurement of both portal vein and mesenteric lymph partitions can provide direct evidence for the different absorbabilities and pharmacokinetic profiles in each absorption system. Our study presents a methodology using the cannulated ileocolic vein, common bile duct, mesenteric lymphatic duct, jugular vein, and duodenum for biological fluid sampling and drug administration in rats. This experimental model offers advantages of normal lymph flow rate and gastric emptying and allows period sampling of mesenteric lymph fluid, portal blood, and jugular blood from cannulated rats.

There are a number of controversies as to whether flavanoids or flavanoid glycosides can be directly absorbed through intestinal cells to enter the circulation[19] or whether they are cleaved by the enzymes of the intestinal bacteria in the small intestinal prior to absorption or hydrolyzed by the intestinal microflora of the large intestine. It had been assumed that the flavanoid glycosides cannot be absorbed from the small intestine and cleavage at the central heterocyclic ring by the enzymes of the intestinal bacteria, which would effectively change the antioxidant activity by delaying it until the substances arrive at the microflora of the large intestine. For the absorption of naringin and naringenin, it has been reported that naringenin must be absorbed as the aglycone after cleavage of naringin by intestinal bacteria, and after absorption, naringin

must be hydrolyzed to naringenin or other naringin disaccharides and then conjugated with glucuronic acid prior to excretion. In general, the metabolites are formed by microbial action, and if the antibiotics were dosed prior to flavanoid uptake, then the metabolites would not be detected in germ-free rats.[34,35] More studies have elucidated the biotransformation of naringenin-7-rhamnoglucoside (naringin) and naringenin in normal rats with microbial action by hydroxylation.[36] To investigate the portal vein in lymphatic absorption and hepatobiliary excretion, the specific pathogen-free animals were used in this experiment.

A Chinese medicine called Si-Ni-San extract was given orally with a dose of 40 g/kg (Si-Ni-San equivalent to 21.5 mg/g for naringin). The pharmacokinetic data demonstrated that the C_{max} values were 368.9 ± 195.3 and 1034 ± 402.0 ng/mL for naringin and naringenin, respectively.[37] A *Fructus aurantii* extract has been investigated after oral administration to rats at dose of 8 mL/kg (an extract of concentration equivalent to 0.4 g/mL *F. aurantii*). The contents of naringin and naringenin in the extract were 0.812 and 0.026 mg/g, respectively. The pharmacokinetic data showed that the C_{max} values were 279.1 ± 53.83 and 1088 ± 198.7 ng/mL for naringin and naringenin, respectively.[38] These researches provide diverse pharmacokinetic data which may suggest that a complicated absorption mechanism occurs in the gastrointestinal tract for naringin and naringenin. Our study presents a methodology using the cannulated ileocolic vein, common bile duct, mesenteric lymphatic duct, jugular vein, and duodenum for biological fluid sampling and drug administration in rats. This multiple sampling model on the same animal can be described to reduce interanimal bias and enhance consistency in a single experimental animal. Our pharmacokinetic data demonstrate that the trends of AUC for naringin in bile and portal vein are much higher than those in jugular vein and lymph fluid, which suggests that the portal vein is the major absorption route and that hepatobiliary excretion happens in naringin (Table 2). However, the portal vein AUC of the aglycone naringenin is 4-fold greater than that of bile, which suggests that the portal vein is still the main route for naringenin absorption but the amount of hepatobiliary excretion is much less than that of glycoside naringin (Table 3). The biotransformation commonly occurs in the intestinal hepatic system, and our experimental data clearly demonstrate that the enterohepatic circulation of naringin is higher than that of naringenin.

The present study involved cannulated mesenteric lymphatic/portal vein/jugular vein/bile duct/duodenum, which can measure the concentration of drug directly after intestinal absorption. In it, we have indicated those flavanoids and their glycosides, naringin and naringenin, can be detected in portal plasma, mesenteric lymph fluid, jugular plasma, and bile as their original form after intraduodenal administration of each. The significantly higher concentration of both drugs in portal plasma than in mesenteric lymph fluid demonstrates that naringin and naringenin are much more likely to be absorbed into portal plasma than through lymph and can be absorbed directly by the gastrointestinal tract without hydrolysis or cleavage before absorption. Furthermore, the high concentration of naringin and naringenin in jugular plasma also demonstrates that both analytes not only are able to enter systemic circulation without hepatic first-pass effect via lymphatic absorption but may also be transported into the systemic circulation after hepatic metabolism via portal absorption.

In this study, an unconscious, mesenteric lymphatic/portal vein/jugular vein/bile duct/duodenum-cannulated rat model was successfully developed to investigate the partition of lymphatic and portal vein absorption with intraduodenally administered drugs. This study integrates the absorption and pharmacokinetics of naringin and naringenin in intestinal absorption, lymphatic absorption, systemic circulation, and bile excretion. The results suggest that the maximum concentration and AUC of both analytes in portal plasma were significantly higher than those in mesenteric lymph fluid. These phenomena indicate that both naringin and naringenin could be absorbed mainly by portal blood, rather than by mesenteric lymph fluid, and could be eliminated through bile excretion, with only a little entering systemic circulation after hepatic metabolism.

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Notes

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