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ARTICLE

Lymphatic Absorption of Quercetin and Rutin in Rat and Their Pharmacokinetics in Systemic Plasma

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Substances and macromolecules absorbed by the lymphatic system avoid hepatic first-pass effect and directly enter the blood circulation system. In this study, an anesthetized, mesenteric lymphatic/duodenum-cannulated rat model was used to investigate the role of lymphatic absorption with intraduodenally administered drugs. Quercetin and rutin were administered, respectively, at dosages of 30 and 300 mg/kg intraduodenally. Lymph and plasma samples were collected every 30 min. These samples were prepared by protein precipitation and then analyzed by high-performance liquid chromatography with a photodiode array detector (HPLC-PDA) and verified by LC tandem mass spectrometry (LC-MS/MS). Quercetin was separated by a C18 reversed-phase column, and rutin was separated by a phenyl reverse-phase column. Pharmacokinetic parameters were calculated using the software WinNonlin Standard Edition Version. The maximum concentration (C_{max}) of quercetin recovered in lymph, 1.97 ± 0.96 μg/mL, was about 5-fold higher than that in plasma, 0.41 ± 0.08 μg/mL. The time to reach the highest concentration (T_{max}) of quercetin in lymph was 30 min longer than that in plasma. The maximun concentration (C_{max}) of rutin recovered in lymph, 0.86 ± 0.13 μg/mL, was slightly lower than that in plasma, 1.35 ± 0.37 μg/mL. The area under curve (AUC) of rutin recovered in lymph, 359 ± 41 min μg/mL, was about 2-fold higher than the AUC of rutin in plasma, 150 ± 22 min μg/mL. This phenomenon was due to the milder concentration decline of rutin in the lymphatic system. These results demonstrate the pharmacokinetic data of lymphatic and systemic absorption after intraduodenally administered quercetin and rutin. It is also the first report revealing the lymphatic absorption of rutin. Although both quercetin and rutin are absorbed and transported mainly via the blood circulation system, the AUC of these two drugs in lymph fluid appeared higher than their respective AUC in plasma.

KEYWORDS: Herbal ingredient; intraduodenal administration; lymphatic absorption; pharmacokinetics; quercetin; rutin

INTRODUCTION

The lymphatic system is part of the body's fluid circulation system, composed of nodules and ducts that are recognized as lymph nodes and lymph ducts. Its functions include immune system regulation and maintenance, extracellular fluid absorption and recirculation, specific cellular and macromolecular particle transportation, nutrient absorption from intestinal tracts, and so on (*1*). Of late, the subject of lymphatic absorption has gained more and more attention in the fields of pharmacology and nutrition (*2*,*3*). This is due to the role of the lymphatic system not only as a route of nutrient absorption and macroparticle uptake but also as a way for molecules to be absorbed into the body while avoiding the hepatic first-pass effect (*4*). Substances absorbed by lacteal ducts enter the mesenteric lymph duct and then pass into the cisterna chyli, where lymph fluid from the intestines and lower body merge together. From the cisterna chyli lymph fluid flows upward into the thoracic duct. Lymph will enter the blood circulation at the junction of the subclavian vein and the internal jugular vein (*5*). By following this route, drugs or other exogenous substances may enter whole-body blood circulation without hepatic fist-pass metabolism via lymphatic absorption. This is important for drugs and substances that are easily metabolized by hepatic enzymes.

Research about lymphatic absorption has discovered that lipophilic, macromolecular substances are more likely to be transported via the lymphatic system (*6*−*9*). As an example, halofantrine is the most well-known drug that is mainly transported through the lymphatic system (*10*). This discovery provided a new vision for researchers, showing that other than blood capillaries there is another pathway for absorbing drugs administered orally. Topics of pharmacokinetic study on lymphatic absorption of orally administered drugs are emerging as new fields of investigation, and gastrointestinal lymphatic absorption indeed may be an alternative for drug administration in the future. However, due to the complex nature of the lymphatic

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system, there are only a few papers discussing pharmacokinetic profiles of the lymphatic system to date (*11*).

According to Murota and Terao (*12*), quercetin is proven to be transported both by intestinal capillaries and by lacteal ducts. Because of this characteristic of quercetin, it was chosen as the test drug to investigate the pharmacokinetic profile of lymphatic absorption compared with intestinal capillary absorption. Lymph fluid and plasma of rats collected at fixed time points were then analyzed using HPLC-PDA. With the quercetin concentrations detected in lymph fluid and plasma samples; pharmacokinetic profiles of quercetin in plasma and lymph fluid were calculated by WinNonlin software using a noncompartmental model.

Rutin is the glycoside of quercetin and is used in ethnomedicine and veterinary medicine for lymphedema (*13*, *14*) and chylothorax treatment (*15*−*17*). Although rutin has been used for clinical treatment, and it is available "over the counter", the opinion on its absorption remains controversial. Some researchers claim that intact rutin could be directly absorbed through intestinal cells (*18*, *19*), whereas others assert that hydrolysis by intestinal microflora is required for rutin absorption (*20*−*23*). If the process of hydrolysis to quercetin is essential to rutin absorption, quercetin should also be effective in chylothorax treatment, but there are no references discussing the application of quercetin in chylothorax or lymphedema treatment. Therefore, on the basis of the therapeutic effect of rutin, we supposed that intact rutin could be absorbed into the body via pathways other than capillaries and finally reach the target tissue. Intestinal lymphatic absorption is one possible pathway for rutin absorption and transport into the body. The unconscious mesenteric lymphatic-cannulated rat model was used to explore the lymphatic absorption of quercetin and rutin in rat and their pharmacokinetics in systemic plasma after intraduodenal rutin administration.

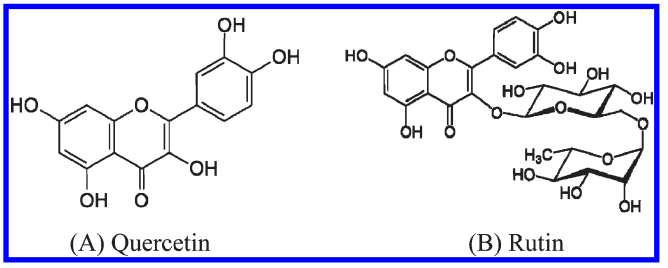

Figure 1. Chemical structures of (**A**) quercetin and (**B**) rutin.

MATERIALS AND METHODS

Chemicals. Quercetin dihydrate and rutin (**Figure 1**) were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol and acetonitrile of HPLC grade were obtained from E. Merck (Darmstadt, Germany). Triply deionized water generated from a Millipore Milli-Q ultrapure water system (Bedford, MA) was used for all preparations.

Animals. Male adult Sprague−Dawley rats weighing from 250 to 300 g were obtained from the Laboratory Animal Center of National Yang-Ming University. All animal care and husbandry procedures were in accordance with the Guide for the Care and Use of Laboratory Animals, and the animal experiment protocols were reviewed and approved by the institutional animal experimentation committee of National Yang-Ming University. Animals were housed in a temperature-controlled room under standard conditions of light and dark cycles, with food pellets and water given ad libitum.

Surgical Procedures. Animals were fasted for 12 h before surgery. Sesame oil (1 mL per rat) was fed 30 min prior to surgery, in order to visualize the mesenteric lymph duct. The rats were anesthetized by intraperitoneal injection of 1 g/mL urethane and 0.1 g/mL α-chloralose mixture solution (1 mL/kg). The cannulation protocol was according to the method described by Warshaw (*24*), with slight modification (**Figure 2**). After the right side of the flank was shaved and disinfected, an incision was made in the right abdomen to expose the mesenteric lymph duct and duodenum. The mesenteric lymph duct was milky-white in appearance and adhered to the mesenteric artery. A puncture was made in the mesenteric lymph duct using a 27 gauge needle to facilitate cannulation. The mesenteric lymph duct was cannulated with PE10 tubing (10 cm in length), and the duodenum was cannulated with PE50 tubing (15 cm in length). Cyanoacrylate glue was used for fixing the cannula in place. After cannulation was complete, the incision was closed by suture. The animal was kept warm by a heating pad for the duration of sampling.

Blood/Lymphatic Absorption of Quercetin and Rutin. *Drug Administration and Sampling*. After surgical procedures, the rat was left in a quiet area for 30 min to collect blank samples. Lymph samples

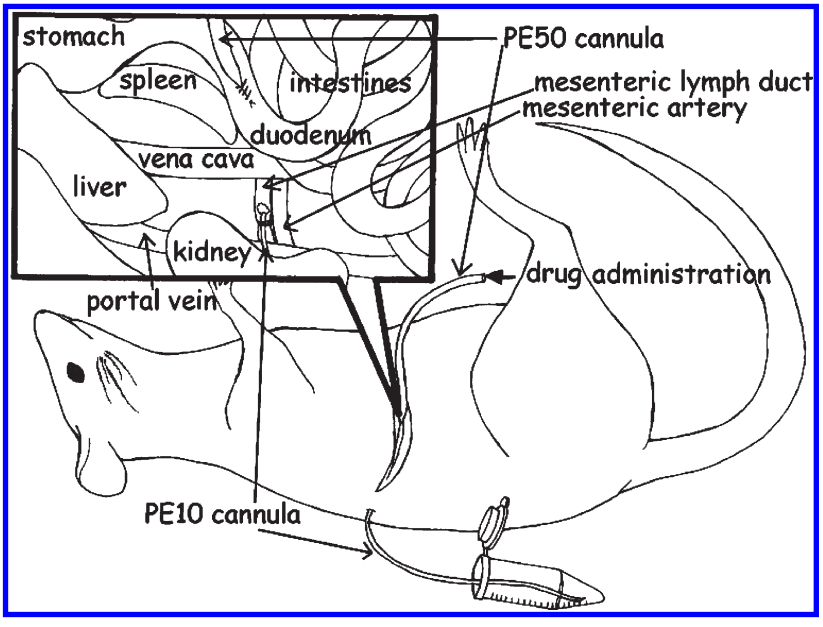

Figure 2. Illustration of the unconscious, mesenteric lymphatic/duodenum-cannulated rat model used in this study. Exteriorization of PE10 tube was done before mesenteric lymph duct catheterization. PE10 cannula was fixed to mesenteric lymph duct by a surgical knot, and the wound was closed by sutures after lymphatic/duodenal cannulation was completed. Quercetin or rutin was administered via PE50 cannula into the duodenum, and mesenteric lymph fluid was collected through PE10 cannula.

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

compd	precursor ion (amu)	product ion (amu)	DP (V)	FP (V)	EP (V)	CE (eV)	CXP (V)	retention time (min)
quercetin	301.2	151.0	−70	−330	−10	−31	−25	4.60
rutin	609.5	300.4	−195	−330	−14	−52	−22	3.25

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

were collected via the lymphatic cannula into a heparin-rinsed Eppendorf, and plasma samples were collected by cardiac puncture using a heparin-rinsed syringe. Quercetin and rutin were dissolved in PEG400/ethanol [4:1 (v/v)] solution, and the concentrations were 30 and 100 mg/mL. The quercetin or rutin solution was administered to the rat at dosage of 1 or 3 mL/kg via duodenal cannula, respectively. After quercetin or rutin administration, lymph and plasma samples were collected every 30 min. The collected biological samples were then stored at −20 °C.

Sample Pretreatment. The procedure of sample pretreatment followed the method described by Murota and Terao (*12*), with minor modification. In brief, 100 μL of acetonitrile/acetic acid 100:5 (v/v) solution was added to 50 μL biological samples and mixed for 3 min. The mixtures were centrifuged at 16110*g* for 10 min at 4 °C, and then the supernatant layer was collected for further analysis.

Liquid Chromatography. The liquid chromatography system was composed of a chromatographic pump (model LC-20AT), a degasser (model DGU-20A5), an autosampler (model SIL-20AC), and a photodiode array detector (model SPD-M20A, Shimadzu, Kyoto, Japan). Quercetin was separated by a C18 reversed-phase column (Synergi Fusion 4u RP-80A, 250 × 4.6 mm, Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile/10 mM NaH_2PO_4 (38:62, v/v, pH 2.75). The flow rate was 1 mL/min. The injection volume was 20 μL. The detection wavelength was 368 nm.

Rutin was separated by a phenyl reversed-phase column (Zorbax SB-phenyl 5 μm, 150 × 4.6 mm, Agilent, Palo Alto, CA). The gradient mobile phase consisted of acetonitrile/10 mM CH_3COONH_4 (pH 3.75): at 0 min, the ratio of organic and aqueous phase was 12:88 (v/v) ; at 5 min, the ratio was 35:65 (v/v); at 12 min, the ratio was 50:50; at 14 min, the ratio was returned to 12:88 for 5 min. The flow rate was 1 mL/min. The injection volume was 20 μL. The detection wavelength was 368 nm.

Method Validation. To assess intraday and interday variabilities, quercetin was assayed at concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2, and 5 μg/mL; rutin was assayed at concentrations of 0.05, 0.1, 0.5, 1, 5, and 10 μg/mL on the same day (six replicates) and on six different days. The accuracy (% bias) was calculated from the mean value of observed concentration (C_{obsd}) and the nominal concentration (C_{nom}) as follows: accuracy (% bias) = $[(C_{obsd} - C_{nom})/C_{nom}] \times 100$. The relative standard deviation (RSD) was calculated from the observed concentrations as follows: precision (% RSD) = [standard deviation (SD)/mean of C_{obsd}] × 100. Accuracy (% bias) and precision (% CV) values were defined as within ±15% (*25*). The lowest concentration of the calibration curve served as the lower limit of quantification (LOQ).

Recovery. The recovery of quercetin in rat plasma and lymph fluid was assessed at three concentrations, 0.05, 0.5, and 5 μg/mL; the rutin concentrations were 0.1, 1, and 10 μg/mL. Recovery (%) was calculated using the formula: recovery = (peak area of analyte in plasma or lymph/peak area of analyte in stock 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). The HPLC system was equipped with a LC binary pump, a microvacuum degasser, an autosampler thermostat, and an autosampler.

HPLC andMS Conditions. Quercetin and rutin were separated on a C18 Luna (5 μm; 4.6 mm × 50 mm) column. Column temperature was maintained at room temperature. The mobile phase composition was a mixture of 10 mM ammonium acetate in 1% formic acid (pH 2.55)/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 0.2 mL/min, and the total run time was 8.0 min for each injection. The injection volume was 5 μL, and the autosampler temperature was set at 4 °C. The injection solvent was 0.1% formic acid/methanol (50:50, v/v).

The 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 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 API3000 mass spectrometer. The flow rate of the syringe pump was 10 μL/min. For precursor and product ion scans, we selected m/z 301.2 → 151.0 for quercetin and 609.5 → 300.4 for rutin in the qualitative analysis. We applied 0.2 μg/mL standard solutions to optimize the mass spectrometer detection conditions in the presence of LC mobile phase. The optimal parameters are shown in **Table 1**. Negative ion multiple reaction monitoring (MRM) mode was used for mass spectrometer detection in the study.

Pharmacokinetics and Statistics. The results are expressed as mean ± standard error 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).

The areas under a plot of drug concentration versus time curves (AUC) were calculated according to the log linear trapezoidal method. The clearances of the drug (Cl/F) were 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 were expressed as $T_{1/2} = 0.693/K$, where K is the first-order rate constant. The volume of distribution (V_d/F) was evaluated as V_d = dose/C_0, where C_0 is the initial plasma concentration. The mean residence time (MRT) was estimated as MRT = AUMC/AUC, where AUMC is the area under the first moment curve. All data are presented as mean ± SEM (*26*).

For comparison of the differences of pharmacokinetic parameters between quercetin and rutin in rat lymph and plasma, Student's t test for paired observations was performed. A P value of <0.05 was considered to be significant. All data are shown as mean ± SEM.

RESULTS AND DISCUSSION

Chromatograms of quercetin samples showed that separation of quercetin from other endogenous substances was achieved with a mobile phase containing 62% 10 mM NaH_2PO_4 (pH 2.75) and 38% acetonitrile. The retention time of quercetin was about 8 min, identified at a UV wavelength of 368 nm. The calibration curve of quercetin in biological samples was constructed over concentration ranges of 0.05−5 μg/mL. The limit of quantification (LOQ) was 0.05 μg/mL. There were no interfering peaks observed during the time quercetin eluted in the chromatograms of blank plasma or lymph fluid.

The recoveries of quercetin at three different concentrations were similar both in plasma and in lymph fluid. Recoveries of quercetin in plasma at concentrations of 0.05, 0.5, and 5 μg/mL were 114.6 ± 1.8, 115.6 ± 1.8, and 113.2 ± 2.3%, respectively. Recoveries of quercetin in lymph fluid at concentrations of 0.05, 0.5, and 5 μg/mL were 107.8 ± 5.9, 101.7 ± 1.4, and 98.5 ± 2.7%, respectively.

The concentration versus time curves of quercetin in plasma and lymph fluid are shown in **Figure 3**. After quercetin administration (30 mg/kg, intraduodenally), the C_{max} of quercetin in plasma (0.41 ± 0.08 μg/mL) occurred at 30 min (T_{max} in plasma). The C_{max} of quercetin in lymph fluid (1.97 ± 0.96 μg/mL) occurred at 60 min (T_{max} in lymph). The C_{max} of quercetin was about 5-fold higher in lymph fluid than in plasma. Quercetin also took longer to reach the highest concentration in lymph fluid than in plasma.

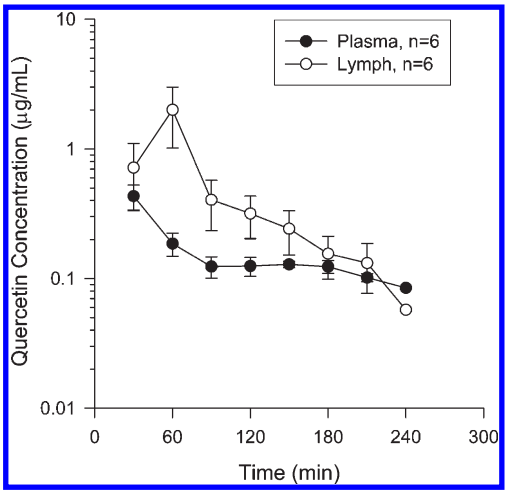

Figure 3. Concentration−time profiles for quercetin in rat plasma and lymph fluid after quercetin intraduodenal administration at a dosage of 30 mg/kg. Data are presented as mean ± standard error mean (n = 6).

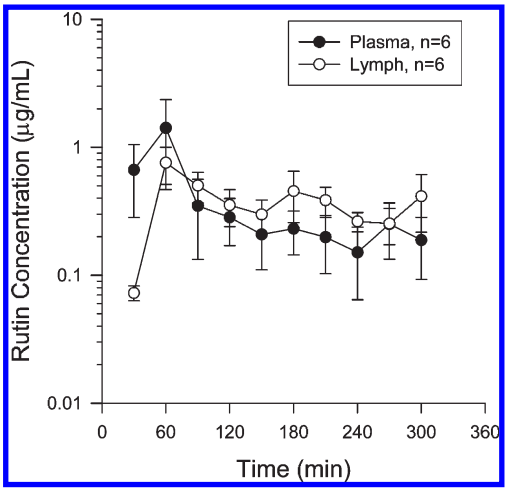

Figure 4. Concentration−time profiles for rutin in rat plasma and lymph fluid after quercetin intraduodenal administration at a dosage of 300 mg/kg. Data are presented as mean ± standard error mean (n = 6).

Table 2. Pharmacokinetic Data of Quercetin) and Rutin in Rat Plasma and Lymph Fluid

	quercetin (30 mg/kg)		rutin (300 mg/kg)	
	plasma	lymph	plasma	lymph
AUC (min μg/mL)	57 ± 6	218 ± 77*	150 ± 22	395 ± 41*
Cl/F (mL/min/kg)	168 ± 19	105 ± 28*	1150 ± 151	241 ± 26*
$T_{1/2}$ (min)	112 ± 31	42 ± 8*	67 ± 8	89 ± 20
C_{max} (μg/mL)	0.41 ± 0.08	1.97 ± 0.96*	1.35 ± 0.37	0.86 ± 0.13
V_d/F (mL/kg)	31.2 ± 7.8	6.4 ± 1.8*	213 ± 23	104 ± 10*
MRT (min)	149 ± 23	79 ± 5*	206 ± 20	318 ± 43*
AUC_{lymph}/ $AUC_{(plasma+lymph)}$	79.3 ± 7.0		72.6 ± 5.0	

[a] Data are expressed as mean ± standard error mean (n = 6). *, P < 0.05 significantly different from plasma of quercetin or rutin.

The pharmacokinetic data of quercetin in plasma and lymph fluid are listed in **Table 2**. These pharmacokinetic data showed that quercetin in lymph fluid had significantly larger AUC, smaller clearance, shorter half-life, higher C_{max}, smaller volume of distribution, and shorter MRT than quercetin in plasma.

Chromatograms of rutin samples from plasma and lymph showed that separation of rutin from other endogenous substances was achieved with a gradient mobile phase containing 10 mM CH_3COONH_4 (pH 3.75) and acetonitrile. The retention time of rutin was about 7.9 min, identified at a UV wavelength of 368 nm. The calibration curve of rutin in biological samples was constructed over concentration ranges of 0.05−10 μg/mL. The LOQ was 0.05 μg/mL. There were no interfering peaks observed during the time rutin eluted in the chromatograms of blank plasma or lymph fluid.

The recoveries of rutin at three different concentrations were similar in both plasma and lymph fluid. Recoveries of rutin in plasma at concentrations of 0.1, 1, and 10 μg/mL were 95.8 ± 6.9, 99.3 ± 5.4, and 93.6 ± 3.3%, respectively. Recoveries of rutin in lymph fluid at concentrations of 0.1, 1, and 10 μg/mL were 93.8 ± 4.4, 98.6 ± 1.1, and 93.2 ± 2.3%, respectively.

The concentration versus time curves of rutin in plasma and lymph fluid are shown in **Figure 4**. After rutin administration (300 mg/kg, intraduodenally), the C_{max} of rutin in plasma (1.35 ± 0.37 μg/mL) occurred at 60 min (T_{max} in plasma). The C_{max} of rutin in lymph fluid (0.86 ± 0.13 μg/mL) occurred at 60 min (T_{max} in lymph). The C_{max} of rutin in plasma was about twice as high as that detected in lymph fluid. The time it took for rutin to reach the T_{max} was the same in lymph fluid and in plasma.

The pharmacokinetic data of rutin in plasma and lymph fluid are listed in **Table 2**. These pharmacokinetic data showed that rutin in lymph fluid had significantly larger AUC, smaller clearance, smaller volume of distribution, and longer MRT than rutin in plasma.

An unconscious rat model was chosen because of its convenience during manipulation, ease of sampling lymph fluid and plasma simultaneously, and higher success rate than a conscious rat model. However, the disadvantages of an unconscious rat model include fewer representatives of actual physiological conditions, slower metabolic rate, and less output of lymph fluid (*4*, *14*, *27*). The mesenteric lymph duct was chosen as the site of cannulation due to the fact that quercetin absorbed from the intestines would directly pass through this site, whereas cannulation of the cisterna chyli would collect lymph fluid from both the intestines and all other parts of the lower body (*28*).

In this study, both quercetin and rutin were dissolved in a PEG400/ethanol [4:1 (v/v)] solution due to favorable properties and applications of PEG. When small-molecule drugs dissolve in PEG solution, they will undergo pegylation and form a conjugated type drug, which can improve drug solubility, enhance permeability through biological barriers, increase longevity in bloodstream, and provide a controlled release (*29*). The purpose of our experiment was to test the permeability of quercetin and rutin in plasma and lymphatic absorption system. Primarily, the PEG400/ethanol (4:1) solution was used to enhance the solubility of quercetin and rutin. To minimize the adverse effects of the vehicle, only a small amount of vehicle (1 or 3 mL/kg) was used.

The doses of administered quercetin and rutin were chosen on the basis of previous studies and clinical references. The administered dose of quercetin, 30 mg/kg, was in accordance with the doses reported by Murota and Terao (10 mg/kg) (*12*) and by Piskula and Terao (50 mg/kg) (*30*). The dosage of quercetin in clinical application is approximately from 0.5 to 15 mg/kg (*31*). The dosage we chose for rutin in this study fell within the limits of a standard clinical dose. In veterinary medicine, the dose of rutin used to treat chylothorax ranges from 100 to 500 mg/kg (*15*, *17*). Therefore, in this study, a total dose of rutin was chosen as 300 mg/kg, but due to its poor solubility, we could dissolve it only at 100 mg/mL in the vehicle, and thus rutin was administered at a dosage of 3 mL/kg intraduodenally.

The maximum concentration of quercetin detected in lymph fluid in this study was 2−3 times higher than that reported by Murota and Terao (*12*). This might be due to a difference between

a conscious and an unconscious model, different cannulation sites of mesenteric lymphatic versus cisterna chyli, different drug administration sites (stomach or duodemun), or different dosages. Murota also mentioned that the highest concentrations of quercetin metabolites are higher than itself in lymph fluid when compared to another paper, and Murota suggests that quercetin is transported mainly through blood circulation (*12*, *32*).

In this study, we examined the systemic and lymphatic absorbability of quercetin and rutin in rats. Although no metabolites of quercetin or rutin were analyzed, drug concentrations were tested simultaneously in both plasma and lymph fluid. Simultaneous measurement of both lymph and plasma concentrations provides a clear platform for comparison of the different pharmacokinetic profiles of a drug in these two different tissues of the body. Furthermore, LC-MS/MS was used for confirmation. Taken together, simultaneous sampling and LC-MS/MS confirmation in sample analysis, there should be sufficient reduction of inaccuracy.

Figures 5 and **6** show the negative ion MRM chromatograms of rat plasma and lymph. Under ESI conditions, quercetin gave $[M - H]^-$ at m/z 301.2 and rutin gave $[M - H]^-$ at m/z 609.5. The precursor/product ion pairs at m/z 301.2/151.0 and 609.5/300.4 were selected in the MRM mode for qualitative analysis of quercetin and rutin. Compared with HPLC-PDA data, after quercetin or rutin administration, existence of both drugs in rat plasma and lymph was reconfirmed. The highest concentration of quercetin was higher in mesenteric lymph fluid, and it also took longer to reach the highest concentration of quercetin in lymph fluid.

The rat plasma samples analyzed in this experiment were all collected by cardiac puncture, which is blood that has undergone hepatic first-pass metabolism. Previous papers also state that concentrations of unmetabolized quercetin were higher in mesenteric lymph fluid than in plasma (*12*, *32*). The smaller volume of the lymphatic system when compared with the blood circulatory system might be a reason for the higher concentration of quercetin in lymph fluid. There were multiple factors determining the concentration of quercetin in lymph fluid, and research still needs to be done to reveal the detailed characteristics of lymphatic absorption in relation to pharmacokinetics. The slower absorption rate of the mesenteric lymphatic system than of capillaries in intestinal villi was also observed in this experiment.

The lymphatic absorption rates, defined as the ratio of $AUC_{lymph}/AUC_{(plasma+lymph)}$, of quercetin and rutin are 79.3 ± 7 and $72.6 \pm 5\%$, respectively. The high lymphatic absorption rate of quercetin and rutin may be due to the smaller volume of the lymphatic system and bypassing the hepatic first-pass effect. The results indicate that the lymphatic system has a smaller volume of drug distribution than the blood circulatory system, which is in agreement with the higher concentration of quercetin detected in mesenteric lymph fluid. There are papers stating that administration with lipids or emulsifiers enhances the lymphatic absorption of quercetin in the intestinal tracts (*33*, *34*). The pegylated dosage form facilitates lymphatic absorption and transport. The clearance of quercetin is greater in plasma than in lymph fluid, and the elimination half-life and MRT of quercetin in plasma are longer than in lymph fluid. This phenomenon can partially be attributed to the much larger volume of the blood circulatory system compared to the volume of the lymph system.

It remains controversial whether intact rutin can be absorbed directly through intestinal cells, ending up in the blood. Some researchers assert that hydrolysis by intestinal microflora is required for rutin absorption (*20*−*22*), whereas others claim that rutin can be directly absorbed through intestinal cells (*18*, *19*). Our results show that rutin can be detected in plasma and in lymph fluid after intraduodenal rutin administration. The highest concentration of rutin detected in plasma was $1.35 \pm 0.37\ \mu g/mL$,

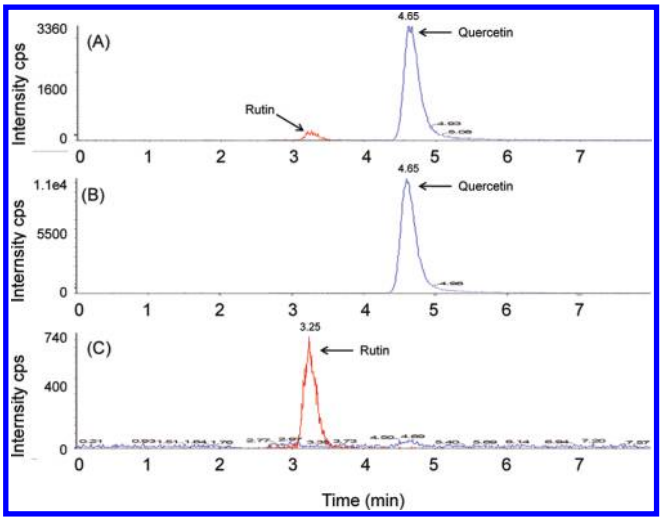

Figure 5. Negative ion multiple reaction monitoring chromatograms of rat plasma at m/z 301.2 → 151.0 for quercetin and 609.5 → 300.4 for rutin obtained from (**A**) blank plasma containing 0.2 μg/mL rutin and quercetin, (**B**) plasma sample collected at 60 min after quercetin (30 mg/kg) administration, and (**C**) plasma sample collected at 60 min after rutin (300 mg/kg) administration. (The arrow indicates the position of rutin and quercetin.)

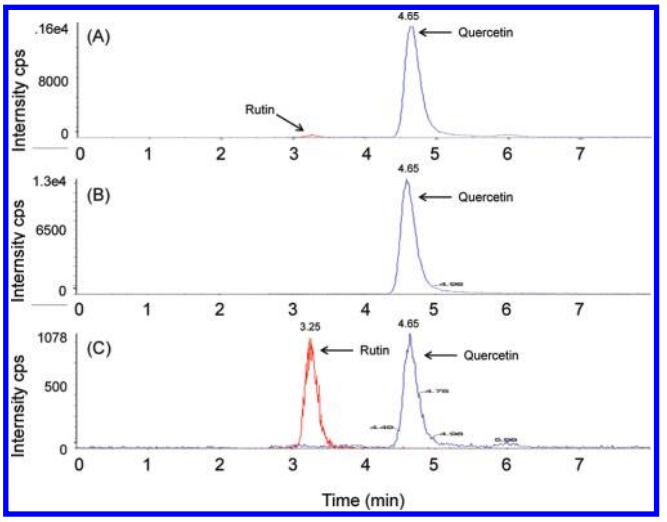

Figure 6. Negative ion multiple reaction monitoring chromatograms of rat plasma at m/z 301.2 → 151.0 for quercetin and 609.5 → 300.4 for rutin obtained from (**A**) blank lymph containing 0.2 μg/mL rutin and quercetin, (**B**) lymph sample collected at 60 min after quercetin (30 mg/kg) administration, and (**C**) lymph sample collected at 60 min after rutin (300 mg/kg) administration. (The arrow indicates the position of rutin and quercetin.)

which was higher than that in lymph fluid ($0.86 \pm 0.13\ \mu g/mL$). The result demonstrates that rutin is more likely to be absorbed into plasma than into lymph fluid. This is in agreement with the higher hydrophilicity of rutin compared to quercetin. Although the C_{max} of rutin in plasma is higher, the AUC of rutin in plasma is significantly smaller than that in lymph fluid due to the smaller clearance rate and longer half-life of rutin in lymph. The time to reach the highest concentration in plasma and in lymph is 60 min after administration. Compared to quercetin, the absorption of rutin happens more slowly in plasma. The administered dose of rutin was 300 mg/kg, whereas the administered dose of quercetin was 30 mg/kg, but the concentrations of rutin and quercetin detected in plasma and in lymph fluid did not directly reflect this

difference in dosage. Thus, rutin may be a substance that is more difficult for intestinal cells to absorb than quercetin.

The pharmacokinetic study of rutin shows that the lymph system has a smaller volume of drug distribution than the blood circulatory system. The plasma protein binding rate of quercetin is greater than that of rutin. According to Bauer-Staeb and Gugler, the plasma protein binding rate of rutin is about 70% (*35*) and that of quercetin is > 98% (*36*). The plasma protein binding rate could account for such a difference in V_d/F between plasma and lymph. The V_d/F of rutin is larger than quercetin's in plasma and lymph fluid. Rutin's larger V_d/F than quercetin's leads to the speculation that rutin is more likely than quercetin to undergo conjugation or hydrolysis in vivo, despite a lower plasma protein binding rate.

This is the first study that compares the pharmacokinetic data of intraduodenally administered quercetin in lymph fluid with that in plasma, and it is also the first to reveal the lymphatic absorption of rutin. The results prove that intact rutin can be absorbed directly through intestinal cells. Although both quercetin and rutin are absorbed and transported mainly via blood circulation, the concentrations of these two drugs appear higher in lymph than in plasma.

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