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# Analysis of Biliary Excretion of Icariin in Rats

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Icariin is a bioactive herbal ingredient isolated from Epimedii Herba. This study evaluates the distribution of icariin in rats by microdialysis sampling and high-performance liquid chromatography with ultraviolet detection (HPLC-UV). Microdialysis probes were simultaneously placed in the jugular vein, brain striatum, and bile duct of each anesthetized rat for sampling after the administration of icariin (dose = 10 or 20 mg/kg) via the femoral vein. The role of P-glycoprotein (P-gp) on icariin distribution was assessed by pretreatment with cyclosporine (CsA, dose = 20 mg/kg). This study is the first report of the biliary excretion of icarin in rats, defined as the blood-to-bile distribution ($k$ value), calculated by dividing the area under the concentration−time curve (AUC) of icariin in bile by that in blood ($k = AUC_{bile}/AUC_{blood}$). The $k$ values were 19.0 ± 5.9 and 18.8 ± 3.8 at the doses of 10 and 20 mg/kg, respectively. The decreased biliary excretion of icariin due to pretreatment with CsA was evidenced by the reduced $k$ values (18.8 ± 3.8 vs 9.9 ± 1.9, $p$ = 0.005). This work demonstrates that biliary excretion is the major elimination pathway for icariin disposition and that transporters, such as P-gp, might be related to icariin's biliary excretion.

**KEYWORDS:** Icariin; biliary excretion; P-glycoprotein; microdialysis

## INTRODUCTION

Icariin (**Figure 1**) is a typical flavonol glycoside extracted from horny goat weed, which belongs to the *Epimedium* genus (Berberidaceae). Epimedii Herba has been used in traditional Chinese medicines over centuries to treat a wide range of diseases, especially to nourish the kidney viscera and reinforce yang, which refers to its tonic actions, such as delaying aging and the improvement of sexual function (*1*, *2*). Icariin has been determined to be the main active component of Epimedii Herba (*3*, *4*). In recent studies, icariin was found to inhibit all three phosphodiesterase-5 isoforms by approximately 50% ($IC_{50}$), which is around 3 times that of zaprinast (a phosphodiesterase-5-specific inhibitor). In addition, icariin can enhance cyclic guanosine monophosphate levels in sodium nitroprusside-treated cavernous smooth muscle cells (*5*). Chronic oral administration of icariin has been shown to have an antidepressant-like effect in the mouse (*1*, *6*). More recent papers have clarified the effects of icariin on bone mass, osteoblast anabolism, and osteoblastic functions (*7*−*9*).

Although a number of diverse pharmacological activities of icariin have been discovered, its pharmacokinetic profile is limited to the results obtained by oral administration or total form icariin concentrations in plasma (*10*−*14*). One paper has studied the absorption of icariin and other prenylated flavonoids in the human intestinal Caco-2 model and the perfused rat intestinal model, finding that the efflux of icariin is significantly reduced only by the P-glycoprotein (P-gp) inhibitor verapamil (*15*). However, little is known about icariin's in vivo distribution. Transporters often play an important role in the distribution of drugs and xenobiotics. P-gp, for example, is a well-known multidrug resistance (MDR) gene MDR1/ABCB1 and ATP-binding cassette (ABC) transporter that exists in several tissue types, including endothelial cells of brain capillaries, apical cells of the gastrointestinal tract, biliary canalicular cells of hepatocytes, and proximal tubular cells of the kidneys (*16*). Thus, it plays an important role in drug absorption, distribution, and excretion and acts as a biological barrier by extruding xenobiotics and

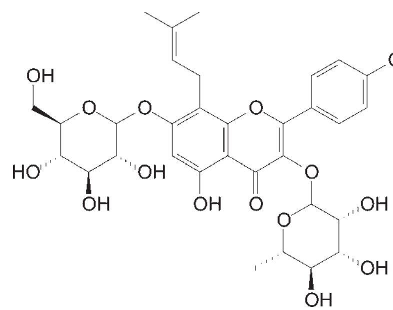


Figure 1. Chemical structure of icariin.

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toxins into the intestinal lumen, bile, and urine and limiting their penetration into brain. The present work evaluates the brain and biliary distribution of icariin using a microdialysis technique for sampling in blood, brain, and bile after a single intravenous dose administration in rats. The microdialysis technique provides pharmacokinetic results with higher temporal resolution because it achieves continuous sampling without excessive body fluid loss from the experimental animal. In addition, microdialysis sampling reduces the number of animals used for distribution studies (*17*). The effect of pretreatment with cyclosporine (CsA), a P-gp inhibitor, on icariin distribution was also evaluated.

## MATERIALS AND METHODS

**Chemicals and Reagents.** Icariin was purified from *Epimedium sagittatum* by one of the authors (L.-C.L.) in the National Research Institute of Chinese Medicine in Taipei, Taiwan (purity = 99% by HPLC). 2-Hydroxypropyl-$\beta$-cyclodextrin (HP-$\beta$-CD) was purchased from Tokyo Chemical Industry (Tokyo, Japan). CsA was obtained from Novartis Pharm. AG (Basle, Switzerland). Liquid chromatographic grade solvent and reagents were purchased from E. Merck (Darmstadt, Germany). Water prepared by the Milli-Q system (Millipore, Bedford, MA) was used for all preparations.

**Liquid Chromatography.** The HPLC system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN), an autosampler (CMA 200, Stockholm, Sweden) equipped with a 20 $\mu$L sample loop, and an UV−vis detector (Varian, Walnut Creek, CA). Icariin and dialysates were separated using a LiChrospher 100 reversed-phase $C_{18}$ column (250 × 4.6 mm i.d.; 5 $\mu$m, Merck) maintained at ambient temperature (25 ± 1 °C). The mobile phase was composed of acetonitrile/10 mM $KH_2PO_4$ (29:71, v/v, pH 4.0 adjusted with orthophosphoric acid), and the flow rate of the mobile phase was 1 mL/min. The mobile phase was filtered through a 0.45 $\mu$m Millipore membrane filter and degassed prior to use. The UV detection wavelength was set at 270 nm, and output data from the detector were integrated via an EZChrom (Scientific Software, San Ramon, CA) chromatographic data system.

**Sample Preparation and Method Validation.** Standard stock solutions were prepared by dissolving icariin in methanol (1 mg/mL). Calibration curves were generated by spiking standard solutions in blank rat dialysates of blood, brain, and bile. Microdialysis samples were analyzed without further preparation. The intra-assay and interassay variabilities were determined at concentrations of 0.05−5 $\mu$g/mL for brain and blood dialysate and of 0.5−10 $\mu$g/mL for bile dialysate. The intra- and interassay variabilities were determined by quantitating six replicates using the HPLC method described above on the same day and six consecutive days, respectively. All calibration curves were required to have a coefficient of determination ($r^2$) of at least 0.995. The limit of quantification (LOQ) was defined as the lowest tested concentration at which the relative standard deviation (RSD) was < 20%. Accuracy and precision values for the lowest acceptable reproducibility concentrations were defined as being within ±20% (*18*).

Plasma samples (60 $\mu$L) were prepared by protein precipitation using 3-fold volume (v/v) methanol containing internal standard (*N*-phenylacetamide, 3.2 $\mu$g/mL). The samples were vortex-mixed for 1 min and centrifuged at 10000*g* for 10 min. The supernatant was filtered using a 0.45 $\mu$m syringe filter prior to HPLC analysis. Calibration standards of plasma samples were prepared by spiking known amounts of icariin into blank rat plasma to give a range of 0.1−20 $\mu$g/mL. Calibration curves were constructed using the internal standard method by least-squares linear regression of the peak area ratios versus the concentrations of icariin. Extraction recovery was performed in triplicate and obtained using eq 1

$$\text{extraction recovery } (\%) = (A_i/A_0) \times 100 \qquad (1)$$

where $A_i$ is the peak area of icariin obtained after the protein precipitation and $A_0$ is the peak area of corresponding concentration of icariin standard solution without the protein precipitation.

**Experimental Animals.** All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of National Yang-Ming University. Male Sprague−Dawley rats (250−350 g) were obtained from the Laboratory Animal Center of National Yang-Ming University, Taipei, Taiwan. Experimental animals were housed in standard rat cages on a 12 h light/dark cycle under institutional guidelines and had free access to food and water until 18 h prior to being used in experiments. The rats were initially anesthetized with urethane (1 g/mL) and α-chloralose (0.1 g/mL; 1 mL/kg, ip) and remained anesthetized during the experimental period as the icariin concentrations in blood, brain, and bile were monitored. After the experimental end point, they were euthanized by overdose of carbon dioxide while still under anesthesia.

**Protein Binding of Icariin.** Icariin (20 mg/kg) was administered intravenously via the femoral vein to rats ($n$ = 6) under anesthesia. Fifteen minutes after drug administration, 3 mL of blood was drawn by cardiac puncture into heparinized tubes and centrifuged at 6000*g* for 10 min to obtain plasma. A 150 $\mu$L aliquot of plasma was used to measure the total form concentration of icariin ($C_t$), and another 150 $\mu$L plasma was further centrifuged by an ultrafiltration tube (Centrifree, Millipore, Bedford, MA) at 10000*g* and 4 °C for 10 min using an Eppendorf 5415R centrifuge (Hamburg, Germany). Protein binding was calculated using eq 2 (*19*)

$$\text{protein binding } (\%) = [(C_t - C_u)/C_t] \times 100 \qquad (2)$$

where $C_t$ is the total drug concentration in the plasma and $C_u$ is the corrected concentration of unbound drug in the filtrate.

**Sampling of Free-Form Icariin in Blood, Brain, and Bile by Microdialysis.** The microdialysis system consisted of a CMA/100 microinjection pump (CMA, Stockholm, Sweden), microdialysis probes, and a CMA/140 fraction collector. The dialysis probes for blood, brain, and bile were made of silica capillary using a concentric design with the tips covered by a dialysis membrane (*17*). The blood microdialysis probe was positioned within the jugular vein/right atrium, whereas the brain microdialysis probe was implanted in the right hippocampus (5.6 mm anterior to bregma, 5.0 mm lateral to midline, and 7.0 mm lower to tip) according to the *Rat Brain Atlas* of Paxinos and Watson (*20*). Ringer's solution ($Na^+$ 147 mM; $Ca^{2+}$ 2.2 mM; $K^+$ 4 mM; pH 7.0) and anticoagulant citrate dextrose (ACD) solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM) as perfusate for brain and blood, respectively, were modified with the addition of ethanol (20%, v/v) and HP-$\beta$-CD (3%, w/v) to improve the extraction efficiency. The bile duct microdialysis probe was constructed in our laboratory on the basis of the design described in previous papers (*21*−*23*). The catheterization of the bile duct and the connection of the bile probe to the bile duct cannula were performed as described previously (*17*, *24*). The bile microdialysis probe was then perfused with Ringer's solution. Animals were randomized to three groups ($n$ = 6 for each group), two of which received 10 or 20 mg/kg icariin only, whereas the third group was pretreated with CsA 20 mg/kg 10 min before icariin administration (20 mg/kg). Drug was administered via the femoral vein after a 2 h postsurgical stabilization period following the implantation of microdialysis probes. The flow rate of perfusate was 2.6 $\mu$L/min, and samples were collected every 10 min.

**Microdialysis Recovery.** The microdialysis recovery of icariin was estimated in vitro (*25*). Briefly, the dialysis membrane of the brain and blood microdialysis probes ($n$ = 3 for each type) were immersed in the Ringer's and ACD solution containing icariin (5 and 10 $\mu$g/mL) as a dialysis medium ($C_0$). Then the brain and blood microdialysis probes were perfused under the same conditions as described under Sampling of Free-Form Icariin in Blood, Brain, and Bile by Microdialysis. Bile flow was simulated by delivering the Ringer's solution containing icariin (5 and 10 $\mu$g/mL) at 20 or 40 $\mu$L/min using a syringe infusion pump (KD Scientific 100, Holliston, MA), and the bile probe was perfused with Ringer's solution at 2.6 $\mu$L/min. The relative recovery of icariin across the microdialysis probe ($R_m$) was acquired using eq 3

$$\text{relative recovery } (R_m, \ \%) = (C_d/C_m) \times 100\% \qquad (3)$$

where $C_d$ is icariin concentration in the dialysate and $C_m$ is icariin concentration in the medium. Actual icariin concentration in microdialysate was corrected using eq 4

$$\text{actual icariin concentration} = C_s/R_m \qquad (4)$$

where $C_s$ is icariin concentration in the microdialysis samples and $R_m$ is relative recovery of icariin across the microdialysis probe.

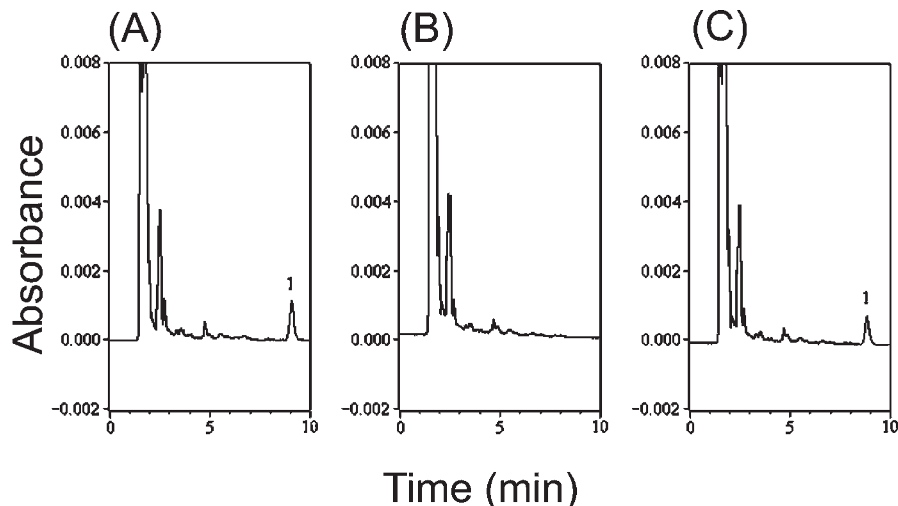


**Figure 2.** Typical chromatograms of (**A**) standard icariin (0.5 $\mu$g/mL) spiked with blank blood dialysate, (**B**) blank blood dialysate, and (**C**) blood dialysate sample containing icariin (0.31 $\mu$g/mL) collected from the jugular vein 10−20 min after icariin administration (20 mg/kg, iv). Peak 1, icariin.

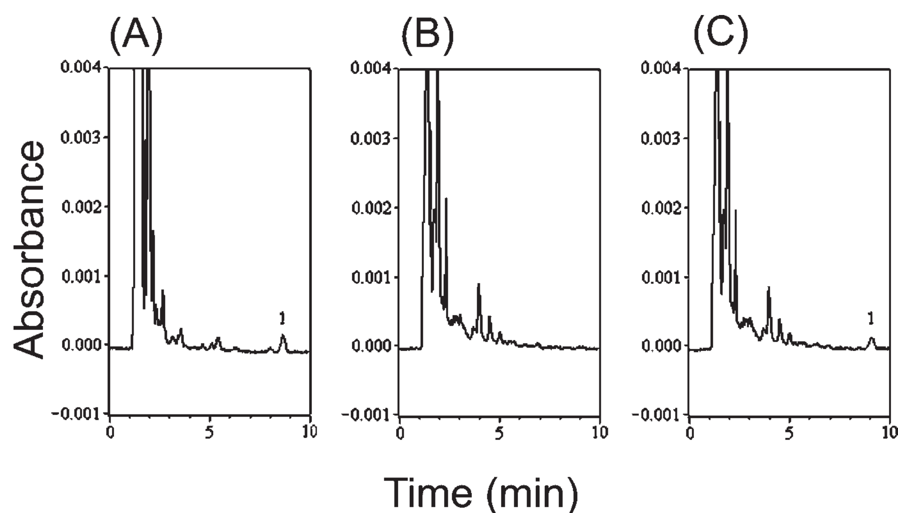


**Figure 3.** Typical chromatograms of (**A**) standard icariin (0.1 $\mu$g/mL) spiked with blank brain dialysate, (**B**) blank brain dialysate, and (**C**) brain dialysate sample containing icariin (0.06 $\mu$g/mL) collected from the jugular vein 10−20 min after icariin administration (20 mg/kg, iv). Peak 1, icariin.

**Data Analysis.** The WinNonlin Standard Edition Version 1.1 (Scientific Consulting Inc., Apex, NC) was used to calculate pharmacokinetic data. An IV-bolus input noncompartmental model was applied to obtain blood pharmacokinetic parameters, and an extravascular input noncompartmental model was applied to obtain bile pharmacokinetic parameters. Results are presented as the mean ± standard deviation of each treatment. Statistics were determined using analysis of variance in the SPSS 18.0 program (SPSS Inc., Chicago, IL). Tukey's honestly significant difference test was used for multiple comparisons of variance. A probability value of $p < 0.05$ was considered to be significant.

## RESULTS AND DISCUSSION

The developed analytical method was used to determine icariin in blood, brain, and bile dialysates (**Figures 2−4**). The intra- and interassay precision values for icariin in blood, brain, and bile dialysates were all < 5.7% bias, and the intra- and interassay accuracies ranged from 92.8 to 108.7% (**Table 1**). The calibration curves for icariin had good linearity ($r^2 > 0.995$) over the ranges of 0.05−5 $\mu$g/mL for brain and blood dialysate and 0.5−10 $\mu$g/mL for bile dialysate. The LOQ values of icariin for brain and blood dialysate samples were estimated to be 0.05 $\mu$g/mL and 0.5 $\mu$g/mL for bile dialysate, respectively. Representative chromatograms of icariin in plasma are shown in **Figure 5**. The plasma calibration curves were considered to be linear ($r^2 > 0.995$) over the ranges of 0.1−20 $\mu$g/mL. The LOQ of icariin in plasma samples was 0.1 $\mu$g/mL. The intra- and interassay precision values for icariin in plasma were all < 14.7% bias, and the intra- and interassay accuracies ranged from 93.9 to 102.4%. The extraction recoveries of protein precipitation for low, middle, and high concentrations were 103.3 ± 7.6, 98.6 ± 4.7, and 102.1 ± 2.3%, respectively.

Plasma levels of icariin obtained at 15 min after icariin (20 mg/kg) administration were 12.0 ± 1.78 $\mu$g/mL (mean ± SD, $n = 6$), and the average binding was estimated to be 94.6 ± 2.2% (mean ± SD, $n = 6$) using ultrafiltration, suggesting a relatively low fraction of free icariin available for microdialysis sampling. In addition, the equilibrium solubilities of icariin at 25 °C were approximately 15, 1931, and 188 mg/L in water, methanol, and ethanol, respectively (*26*). Therefore, modification of the perfusate, such as the addition of HP-$\beta$-CD, ethanol, propylene glycol, or dimethyl sulfoxide, was performed to enhance the probe extraction efficiency (*27*). The perfusate containing ethanol (20%, v/v) and HP-$\beta$-CD (3%, w/v) provided an appropriate recovery for sampling icariin. The bile probe extraction efficiency was almost the same (24.4 ± 0.3 and 25.5 ± 0.2%), although the bile

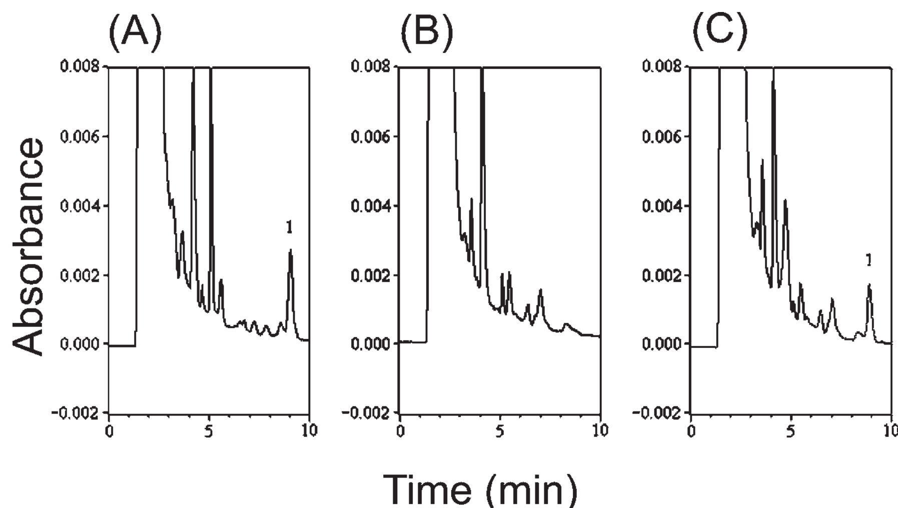


**Figure 4.** Typical chromatograms of (**A**) standard icariin (1.0 $\mu$g/mL) spiked with blank bile dialysate, (**B**) blank bile dialysate, and (**C**) bile dialysate sample containing icariin (0.6 $\mu$g/mL) collected from the bile duct 140−150 min after icariin administration (20 mg/kg, iv). Peak 1, icariin.

**Table 1.** Analytical Accuracy and Precision of Icariin in Blood, Brain, and Bile Microdialysate

| nominal concentration ($\mu$g/mL) | accuracy (%) | precision (%) |
|---|---|---|
| **Blood Dialysate** | | |
| interday | | |
| 0.05 | 107.2 | 3.7 |
| 0.5 | 100.7 | 3.3 |
| 5 | 100.2 | 1.8 |
| intraday | | |
| 0.05 | 108.7 | 1.7 |
| 0.5 | 102.8 | 0.7 |
| 5 | 100.1 | 0.4 |
| **Brain Dialysate** | | |
| interday | | |
| 0.05 | 108.4 | 5.7 |
| 0.5 | 98.1 | 4.2 |
| 5 | 98.6 | 3.9 |
| intraday | | |
| 0.05 | 99.7 | 4.6 |
| 0.5 | 92.8 | 3.7 |
| 5 | 98.5 | 1.8 |
| **Bile Dialysate** | | |
| interday | | |
| 0.5 | 98.5 | 5.2 |
| 5 | 101.4 | 1.8 |
| 10 | 99.7 | 4.4 |
| intraday | | |
| 0.5 | 96.3 | 1.0 |
| 5 | 99.0 | 3.9 |
| 10 | 95.9 | 2.4 |

flow rate varied from 20 to 40 $\mu$L/min, showing that the recovery was independent of bile flow or blood flow (*28*). In addition, the blood and bile probe recoveries were both independent of the icariin concentrations, consistent with the previous paper (*29*). The average in vitro recoveries of icariin from rat blood, brain, and bile microdialysis probes were 3.3 ± 0.4, 7.8 ± 0.4, and 25.0 ± 1.0%, respectively, and each sample concentration was corrected by probe recoveries prior to pharmacokinetic data analysis.

The concentration−time curves of icariin in brain, blood, and bile are shown in **Figure 6**. The dose-related pharmacokinetic behavior of icariin was observed with the doses of 10−20 mg/kg, evidenced by the almost proportional increase of initial concentration ($C_0$) and AUC, together with the unchanged half-life ($t_{1/2}$), clearance (Cl), and volume of distribution ($V_d$) (**Table 2**). The AUC of icariin was elevated after the pretreatment of CsA (280 ± 45 vs 864 ± 150 min $\mu$g/mL, $p < 0.001$). In addition, the CsA pretreatment prolonged the half-life ($t_{1/2}$) (8 ± 3 vs 28 ± 9 min, $p < 0.001$) and decreased the Cl (70.4 ± 11.1 vs 22.7 ± 4.3 mL/min/kg, $p < 0.001$). Flavonoids, such as quercetin, genistein, diosmetin, and luteolin, were recognized as cytochrome P450 monooxygenases (CYPs) substrates and modulators (*30*−*32*). Icariin is a flavonol glycoside that might be metabolized by similar CYPs, and the results suggest that the elevated blood levels of icariin might be caused by enzymatic inhibitions after the administration of CsA.

Icariin was detected in brain microdialysate samples during the first 30 min after drug administration (20 mg/kg icariin, iv) by the current HPLC method (LOQ = 0.05 $\mu$g/mL), as shown in **Figure 3C**. Brain pharmacokinetic parameters were not available due to insufficient and discontinuous time−concentration data, so the concentrations of icariin in samples collected in 20−30 min ($C_{25\ min}$) were selected to roughly assess effects of the pretreatment of CsA. The $C_{25\ min}$ of the 20 mg/kg icariin group was not different from that of the CsA + 20 mg/kg group (1.95 ± 0.17 vs 1.87 ± 0.41 $\mu$g/mL, $n = 3$ for each group, $p = 0.08$). Relatively low extraction efficiency of brain microdialysis probes limits the evaluation of icariin, so a more sensitive quantification method may be necessary to confirm the brain distribution of icariin for future work.

The use of a microdialysis technique for icariin biliary excretion in this study greatly reduces body fluid loss, which is a common problem due to draining the bile of an experimental animal. For biliary excretion, icariin showed an approximately dose-related pharmacokinetic behavior with the doses of 10−20 mg/kg (**Table 2**). The biliary excretion, defined as the blood-to-bile distribution ($k$ value), which was calculated by dividing the AUC of icariin in bile by that in blood ($k = AUC_{bile}/AUC_{blood}$) (*33*), of icariin in rats is first reported in this study. The $k$ values were 19.0 ± 5.9 and 18.8 ± 3.8 for 10 and 20 mg/kg, respectively (**Table 2**). For the elimination of amphipathic, hydrophobic, high molecular weight xenobiotics, biliary excretion is known to be a major pathway, thereby complementing the renal elimination of hydrophilic compounds with low molecular weight (*34*). It has been reported

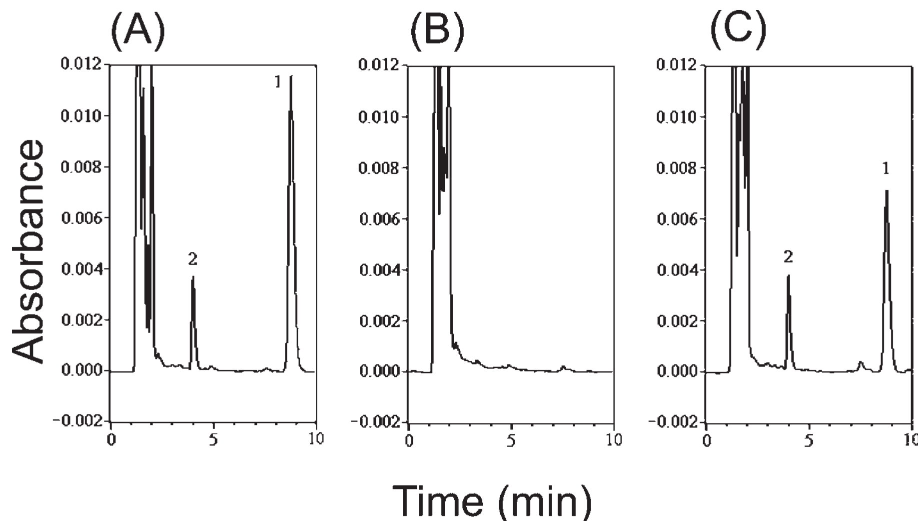


**Figure 5.** Typical chromatograms of (**A**) standard icariin (5.0 μg/mL) spiked with blank plasma, (**B**) blank plasma, and (**C**) plasma sample containing icariin (3.1 μg/mL) collected at 15 min after icariin administration (20 mg/kg, iv). Peak 1, icariin; peak 2, internal standard.

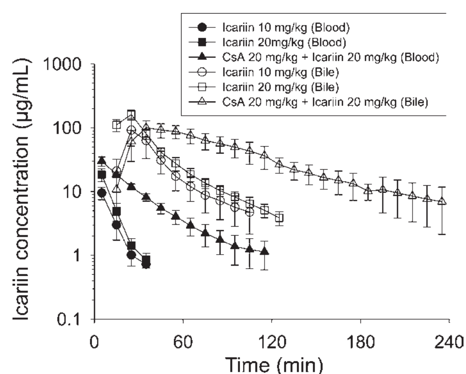


**Figure 6.** Concentration−time profile for icariin in blood and bile dialysate after intravenous administration of icariin at dosages of 10 and 20 mg/kg. Data are represented as mean ± standard deviation from six rats for each group.

that a minimum molecular weight of between 200 and 325 is required for significant biliary excretion in the rat, with 400 and 475 being the corresponding values for the guinea pig and rabbit, respectively (*35*). Biliary excretion of icariin is reasonable, because icariin has a molecular weight of 676 and is not readily soluble in water. A very recent work also indicated that in rats prenylflavonoids should be mostly excreted in the bile (*36*).

Biliary excretion is maintained via driven uphill transport of drugs across cell membranes, which is accomplished by ATP-binding cassette-carriers. The transporters, such as P-gp, multidrug resistance protein 2 (MRP2), or breast cancer resistance protein (BCRP), work in concert with drug metabolism enzymes to eliminate drugs and their metabolites (*37*, *38*). Therefore, this study further investigated the elimination mechanism of icariin in bile by pretreatment with a P-gp inhibitor. The pretreatment of CsA altered the maximum concentration ($C_{max}$) (161.2 ± 27.5 vs 105.0 ± 25.0 μg/mL, $p = 0.02$) and AUC (5146 ± 406 vs 8444 ± 1587 min μg/mL, $p < 0.001$) of icariin. The administration of CsA was expected to inhibit transporter activities to diminish icariin's biliary excretion, but in fact the bile AUC was increased. The increased bile AUC might result from a compensated excretion via bile, because the renal excretion of icariin mediated by P-gp or other transporters was likely inhibited by CsA. The trend of decreased biliary excretion of icariin was evidenced by the

**Table 2.** Estimated Pharmacokinetic Parameters in Rat Blood, Brain, and Bile after Icariin Administration (10 and 20 mg/kg, iv) and Its Interaction with CsA[a]

| parameter | group A | group B | group C |
|---|---|---|---|
| **Blood** | | | |
| $C_0$ (μg/mL) | 17.92 ± 9.31 a | 35.55 ± 9.49 a | 40.35 ± 10.54 |
| $t_{1/2}$ (min) | 7 ± 2 | 8 ± 3 b | 28 ± 9 b |
| AUC (min μg/mL) | 138 ± 33 a | 280 ± 45 ab | 864 ± 150 b |
| Cl (mL/min/kg) | 70.0 ± 13.3 | 70.4 ± 11.1 b | 22.7 ± 4.3 b |
| $V_d$ (mL/kg) | 623 ± 168 | 537 ± 134 | 628 ± 145 |
| **Bile** | | | |
| $C_{max}$ (μg/mL) | 91.9 ± 40.1 a | 161.2 ± 27.5 ab | 105.0 ± 25.0 b |
| $t_{1/2}$ (min) | 27 ± 9 | 29 ± 4 b | 66 ± 27 b |
| AUC (min μg/mL) | 2642 ± 1036 a | 5146 ± 406 ab | 8444 ± 1587 b |
| Cl (mL/min/kg) | 4.1 ± 2.1 | 3.8 ± 0.3 | 2.3 ± 0.6 |
| $V_d$ (mL/kg) | 156 ± 67 | 158 ± 27 | 212 ± 87 |
| $AUC_{bile}/AUC_{blood}$ | 19.0 ± 5.9 | 18.8 ± 3.8 b | 9.9 ± 1.9 b |

[a] Data expressed as mean ± SD ($n = 6$). Group A, icariin (10 mg/kg, iv); group B, icariin (20 mg/kg, iv); group C, CsA (20 mg/kg, iv) + icariin (20 mg/kg, iv). $C_0$, initial concentration; $t_{1/2}$, half-life; AUC, area under the concentration vs time curve; Cl, clearance; $V_d$, volume of distribution; $C_{max}$, maximum concentration; $AUC_{bile}/AUC_{blood}$, ratio between the blood AUC and the bile AUC of each rat. Entries followed by an "a" are significantly different ($p < 0.05$) between groups A and B. Entries followed by a "b" are significantly different ($p < 0.05$) between groups B and C.

reduced $k$ values (18.8 ± 3.8 vs 9.9 ± 1.9, $p = 0.005$). It has been reported that poor bioavailability of icariin and structure-related flavonoids was the result of poor intrinsic permeability and efflux by apical efflux transporters such as MRP2, BCRP, and P-gp in models of human intestinal Caco-2 and the perfused rat intestinal model (*15*). Our results also found the transport of icariin might be governed by transporters, because CsA has been found to interact with a multitude of active transporters such as P-gp, MRP2, and organic anion-transporting polypeptide (OATP) (*39*−*41*), so other transporters might also affect icariin's biliary excretion. In conclusion, microdialysis analysis combined with reversed-phase HPLC-UV has been used to evaluate the distribution of icariin in rats. The results indicate that biliary excretion was the major route for icariin disposition, and the active secretion transport was involved in icariin's biliary excretion.

## ABBREVIATIONS USED

HPLC-UV, high-performance liquid chromatography with ultraviolet detection; P-gp, P-glycoprotein; CsA, cyclosporine; AUC, area under the concentration−time curve; MDR, multidrug resistance; ABC, ATP-binding cassette; HP-$\beta$-CD, 2-hydroxypropyl-$\beta$-cyclodextrin; LOQ, limit of quantification; RSD, relative standard deviation; ACD, anticoagulant citrate dextrose; $C_0$, initial concentration; $t_{1/2}$, half-life; Cl, clearance; $V_d$, volume of distribution; CYPs, cytochrome P450 monooxygenases; MRP2, multidrug resistance protein 2; BCRP, breast cancer resistance protein; $C_{max}$, maximum concentration; OATP, organic anion-transporting polypeptide;

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