JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Naringin in Rat Blood, Brain, Liver, and Bile Using Microdialysis and Its Interaction with Cyclosporin A, a P-Glycoprotein Modulator

TUNG-HU TSAI*

National Research Institute of Chinese Medicine, and Institute of Traditional Medicine, National Yang-Ming University, Taipei 112, Taiwan

To determine naringin levels in various biological fluids, we developed an in vivo microdialysis technique coupled with a microbore HPLC system to investigate the pharmacokinetics of naringin and its interaction with cyclosporin A in rat blood, brain, liver, and bile. After naringin administration, naringin was undetectable in the brain; the distribution ratios of area under the curve (AUC) of liver over that in blood (AUC_{liver}/AUC_{blood}) and of AUC of bile over that in blood (AUC_{bile}/AUC_{blood}) of naringin were 5.39 ± 0.94 and 29.17 ± 3.58, respectively. When cyclosporin A (20 mg/kg) was concomitantly administered with naringin (30 mg/kg), the naringin was detected in brain dialysate, but the distribution ratios of liver and bile showed no statistical difference. These results suggest that naringin was concentrated in the liver and bile by the processes of active transport. The blood−brain barrier penetration of naringin may be enhanced by P-glycoprotein inhibitor; however, the pathway of hepatobiliary excretion of naringin may not be related to the P-glycoprotein.

KEYWORDS: Blood−brain barrier; cyclosporin A; disposition; hepatobiliary excretion; microdialysis; naringin; P-glycoprotein; pharmacokinetics

INTRODUCTION

Naringin is the predominant bioflavonoid in grapefruit and is also suggested as a potential cytochrome P450 inhibitor (*1*). Bioflavonoids derived from citrus fruits have been reported to possess many biological functions, including preventive and therapeutic effects on several diseases. The result of drug−drug interaction with grapefruit was to increase the oral bioavailability of such drugs as midazolam, triazolam, felodipine, and nifedipine. The increase in concentrations of these drugs when administered concomitantly with grapefruit juice was attributed to the inhibition of cytochrome P450 enzymes by naringenin, in which the sugar residue was removed from the grapefruit flavonoid naringin (*2*).

According to classical pharmacokinetics, only the protein-unbound drugs are available for redistribution or to react with receptors and are therefore responsible for activating cellular responses. To determine the pharmacokinetics of the protein-unbound form of naringin in rats, we employed in vivo microdialysis techniques to obtain the protein-free naringin from rat blood, brain, liver, and bile samples derived simultaneously (*3*, *4*). Microdialysis sampling techniques were originally developed to allow in vivo sampling of neurotransmitters released in the brain (*5*, *6*). Techniques have subsequently been extended to encompass pharmacological and pharmacokinetic studies (*7*, *8*). Over the past several years, microdialysis has been increasingly used in various animal experiments, for the in vivo sampling of unbound endogenous or exogenous compounds present in blood, brain, tissue, etc. (*9*, *10*). Sampling by this technique involves continuous perfusion of fluid through microdialysis probes implanted in the appropriate tissue space.

Grapefruit juice bioflavonoids, including quercetin, have been reported to stimulate P-glycoprotein-mediated drug efflux from cultured tumor cells (*11*, *12*). Therefore, it is of particular interest to study the effects of P-glycoprotein inhibitors on naringin distribution. Although several P-glycoprotein inhibitors have been described (e.g., cyclosporin A, verapamil, quinine, nifedipine, etc.), in consideration of the minimal acute peripheral cardiovascular side effects attributable to cyclosporin A, cyclosporin A was employed in this study. Very few studies have been published concerning the effects of P-glycoprotein inhibitor drugs on the pharmacokinetics of naringin.

Naringin has been reported to be eliminated through urinary excretion (*13*). However, the biliary excretion of naringin remains unclear. In addition to investigating the liver level and biliary excretion of naringin, we designed concentric flow-through microdialysis probes for the sampling of naringin from liver and biliary fluid samples following intravenous naringin administration. Therefore, the pharmacokinetics of naringin in rat blood, brain, liver, and bile were evaluated in this study using microdialysis. In addition, the effect of P-glycoprotein on the disposition of naringin was explored.

* Address correspondence to Dr. Tung-Hu Tsai, National Research Institute of Chinese Medicine, 155-1, Li-Nong St. Section 2, Shih-Pai, Taipei 112, Taiwan. Fax: 886-2 2826-4276. Tel.: 886-2 2820-1999, ext 8091. E-mail: thtsai@cma23.nricm.edu.tw.

10.1021/jf020603p CCC: $22.00
Published on Web 10/08/2002

MATERIALS AND METHODS

Chemicals and Reagents. Naringin was purchased from Sigma Chemical Co. (St Louis, MO). Cyclosporin A (Sandimmun) was obtained from Novartis Pharma (Basel, Switzerland). Liquid chromatographic grade solvents and reagents were obtained from E. Merck (Darmstadt, Germany). Triply deionized water (Millipore, Bedford, MA) was used for all preparations.

Animals. All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of the National Research Institute of Chinese Medicine. Male specific pathogen-free Sprague−Dawley rats were obtained from the Laboratory Animal Center of the National Yang-Ming University, Taipei. Before experimentation, animals were allowed a one-week acclimation period at the animal quarters with air conditioning and an automatically controlled photoperiod of 12 h of light daily. Animals had free access to food (laboratory rodent diet no. 5P14, PMI Feeds Inc., Richmond, IN) and water until 18 h prior to experimentation, at which time food was removed. The rats were initially anesthetized with urethane (1 g/mL) and α-chloralose (0.1 g/mL) (1 mL/kg i.p.) and remained anesthetized throughout the experimental period. The femoral vein was exposed for further drug administration. During the experiment, rat body temperature was maintained at 37 °C using a heating pad.

Chromatography. The chromatographic system consisted of a chromatographic pump (BAS PM-80, BAS, West Lafayette, IN), an on-line injector (CMA/160, CMA, Stockholm, Sweden) equipped with a 10-μL sample loop, and a UV/vis detector (Varian, Walnut Creek, CA). Samples obtained were separated using a reverse-phase C18 column (150 × 1 mm; 5 μm). Chromatography was performed at ambient temperature. The mobile phase consisted of citrate buffer (pH 5.0) and acetonitrile−0.1 M ammonium acetate (pH 7.1) (28:72, v/v). This mobile phase was filtered with a 0.22-μm Millipore membrane prior to being used for elution. The chromatographic pump flow rate was set at 0.05 mL/min. The wavelength of naringin was determined to be 283 nm. The output signal from the HPLC−UV/vis detector was recorded using an EZChrom chromatographic data system (Scientific Software, San Ramon, CA).

Microdialysis Experiment. Blood, brain, liver, and bile microdialysis systems consisted of a CMA/100 microinjection pump and the appropriate microdialysis probes. The dialysis probes for blood (10 mm in length), brain (3 mm in length), liver (10 mm in length), and bile (7 cm in length) were made of silica glass capillary tubing arranged in a concentric design (*14*, *15*). The tips of the tubes were covered by a dialysis membrane (150 μm outer diameter with a nominal molecular weight cutoff of 13 000; Spectrum Co., Laguna Hills, CA), and all contact points were cemented with epoxy. To allow adequate time for the epoxy to dry, the probes were made at least 24 h prior to use.

The blood and liver microdialysis probes were positioned within the jugular vein/right atrium and the median lobe of the liver, respectively, and then perfused with anticoagulant dextrose (ACD) solution (3.5 mM citric acid, 7.5 mM sodium citrate, 13.6 mM dextrose) at a flow rate of 1 μL/min using the CMA microinjection pump.

The bile duct microdialysis probes were constructed in-house, according to the design originally described by Scott and Lunte (*16*). This bile microdialysis method has been reported in our previous studies (*17*−*20*).

For brain microdialysis, the rat was mounted on a stereotaxic frame and perfused with Ringer's solution (147 mM Na^+, 2.2 mM Ca^{2+}, 4 mM K^+; pH 7.0). After being washed with Ringer's solution at a flow rate of 2 μL/min, the microdialysis probe was implanted in the right striatum (coordinates: AP 0.4 mm, ML −3.0 mm, DV −7.0 mm) according to the Paxinos and Watson atlas (*21*). The positions of the probes were verified by standard histological procedure at the end of the experiments. This method has been previously reported (*22*).

Drug Administration. After a 2-h postimplantation period, an intravenous dose of drug was administered via the femoral vein. Cyclosporin A (10 mg/mL) was produced by diluting cyclosporin A injectable solution with a 5% dextrose/water solution. Naringin (30 mg/kg) was administered intravenously to the control group ($n = 6$). For the cyclosporin A-treated group ($n = 6$), cyclosporin A (20 mg/kg) was injected via the femoral vein 10 min prior to naringin. The total injection volumes of naringin and cyclosporin A were 1 and 2 mL/kg, respectively. The blood, brain, liver, and bile dialysates were connected to an on-line injector (CMA 160) and a fraction collector (CMA/140). The sampling interval was 10 min for each probe. Blood, brain, liver, and bile dialysates were measured by a microbore HPLC system on the same day as the experiment. To investigate the dose-related phenomenon, a higher dose of naringin (100 mg/kg) was administered.

Recovery of Microdialysis. For in vivo recovery, the blood, brain, liver, and bile microdialysis probes were inserted into the jugular vein, striatum, liver, and bile duct under anesthesia with urethane. Perfusate solution containing naringin was passed through the microdialysis probe at a constant flow rate (1 μL/min) using an infusion pump (CMA/100). Two hours after probe implantation, the perfusate (C_{perf}) and dialysate (C_{dial}) concentrations of naringin were determined by HPLC. The relative recoveries (R_{dial}), in vivo, of naringin across the microdialysis probes inserted in the rat blood, brain, liver, and bile were calculated according to the following equation: $R_{dial} = (C_{perf} - C_{dial})/C_{perf}$.

Pharmacokinetic Application. Naringin microdialysate concentrations (C_m) were converted to unbound concentration (C_u) as follows: $C_u = C_m/R_{dial}$. Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic calculation software WinNonlin Standard Edition Version 1.1 (Scientific Consulting Inc., Apex, NC) by the noncompartmental method (*23*). The areas under the curves (AUCs) from time zero to time infinity were calculated by the trapezoidal rule and extrapolated to time infinity by the addition of AUC_{t-inf}. The AUC values were thus given by the sum of the products of the measured concentrations and the collection time interval, plus the residual area; that is, $AUC = AUC_{0-t} + AUC_{t-inf}$.

Statistics. The results are represented as mean ± standard error of the mean. The statistical analysis was performed by Student's t test (SPSS version 10.0, SPSS, Chicago, IL) to compare between the control (naringin-treated alone) and cyclosporin A-treated groups. The level of significance was set at $p < 0.05$.

RESULTS

Typical chromatograms of naringin sampled from the biological fluids are shown in **Figure 1**. Separation of naringin from endogenous chemicals in biological dialysates was achieved in an optimal mobile phase containing 83% of 25 mM citrate buffer (pH 5.0), 0.1% triethylamine, and 17% acetonitrile. The retention time of naringin was about 6 min, and the peak was identified by its UV spectrum with a Waters 996 photodiode array detector (Waters 2695, Waters, Bedford, MA). The calibration curve of naringin was obtained prior to LC analysis of dialysates over concentration ranges of 0.05−10 μg/mL. This microbore chromatographic system has been validated for both inter- and intraday accuracy, and the determined limits of this precision assay have been deemed acceptable. The limit of quantification was 0.05 μg/mL. To measure the samples with concentrations higher than 10 μg/mL, another calibration curve (10−500 μg/mL) was used. The chromatograms of a blank blood, brain, liver, and bile dialysate indicate that none of the observed peaks interfered with the analyte.

Figure 1A shows the chromatogram of a blood dialysate sample containing naringin (0.23 μg/mL) collected 30 min after naringin administration (30 mg/kg, i.v.). **Figure 1B** shows the chromatogram of a brain striatal dialysate sample containing naringin (0.06 μg/mL) collected 30 min after co-administered with cyclosporin A (20 mg/kg) and naringin (30 mg/kg, i.v.). **Figure 1C** shows the chromatogram of a liver dialysate sample containing naringin (40.08 μg/mL) obtained 30 min after naringin administration (30 mg/kg, i.v.). **Figure 1D** shows the chromatogram of a bile dialysate sample containing naringin (329.03 μg/mL) obtained 30 min after naringin administration (30 mg/kg, i.v.). To optimize the naringin separation from the

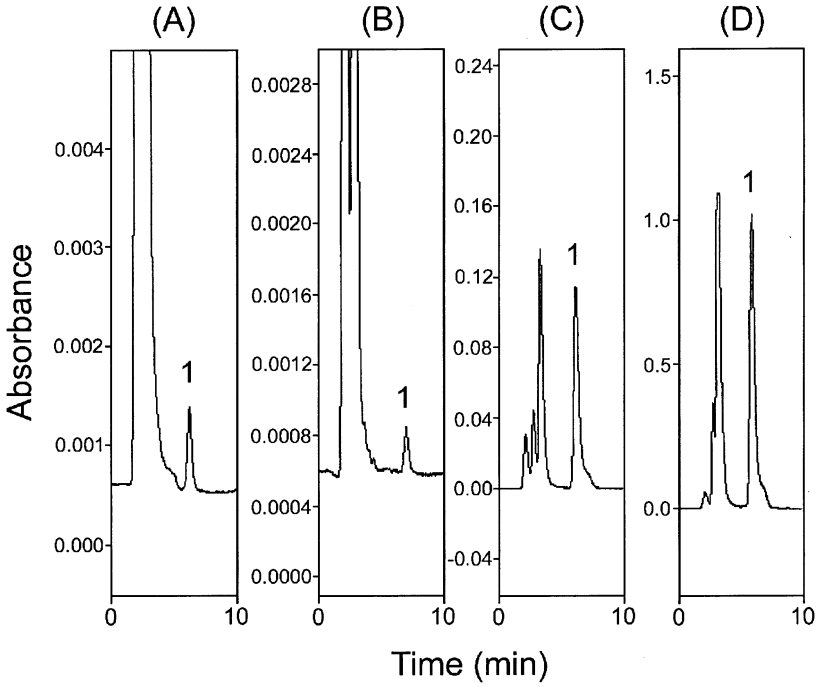

Figure 1. (A) Typical chromatogram showing a blood dialysate sample containing naringin (0.23 μg/mL) collected 30 min after naringin administration (30 mg/kg, i.v.). (B) Chromatogram of a brain striatal dialysate sample containing naringin (0.06 μg/mL) collected 30 min after co-administration of cyclosporin A (20 mg/kg) and naringin (30 mg/kg, i.v.). (C) Chromatogram of a liver dialysate sample containing naringin (47.08 μg/mL) obtained 30 min after naringin administration (30 mg/kg, i.v.). (D) Chromatogram of a bile dialysate sample containing naringin (329.03 μg/mL) obtained 30 min after naringin administration (30 mg/kg, i.v.). 1, Naringin.

dialysate of bile fluid, the acetonitrile concentration and the pH value of the buffer solution were modified from the conditions described in a previous report (*24*).

In vivo recoveries of naringin were 14.7 ± 1.1% (n = 6) for blood (1 μg/mL), 4.3 ± 0.6% (n = 6) for brain (1 μg/mL), 21.4 ± 0.9% (n = 6) for liver (1 μg/mL), and 73.8 ± 1.2% (n = 6) for bile (10 μg/mL). The in vivo recovery (or dialysis efficiency) can be affected by certain factors, mostly physical in nature, such as temperature and perfusion rate. The materials used in the construction of the probe and the final dimensions of the probe can also affect dialysis efficiency. Thus, drug concentration in the biological fluid must be corrected by the recovery.

The concentration versus time curves of naringin in blood, brain, liver, and bile are shown in **Figures 2**−**5**, respectively. The pharmacokinetic profiles of unbound naringin in rat blood, brain, liver, and bile in both control and cyclosporin A-treated groups are presented in **Table 1**. The pharmacokinetic profiles indicate that naringin in blood appears to be dose-related in the dosage ranges of 30−100 mg/kg. Cyclosporin A increased naringin concentration at a dose of 30 mg/kg (**Figure 2**).

It was not possible to detect naringin in the brain at the lower dose of 30 mg/kg; however, concomitant with cyclosporin A, naringin became detectable in the brain at low levels (**Figure 3**). The brain penetration of naringin, defined as the blood-to-brain distribution ratio (k value), was calculated by dividing the naringin AUC in brain by the naringin AUC in blood (k = AUC_{brain}/AUC_{blood}) (*4*). The k value of naringin in brain at a dose of 100 mg/kg is 0.0068, suggesting a poor blood−brain barrier (BBB) penetration of naringin (**Table 2**).

Naringin concentration in the median lobe of the liver was statistically higher than that in blood. The distribution ratios (k value) in liver were 5.39 ± 0.94 and 5.83 ± 0.67 for doses of 30 and 100 mg/kg, respectively (**Figure 4**). Cyclosporin A does not significantly change the distribution ratio in liver at a dose of 30 mg/kg (**Table 1**).

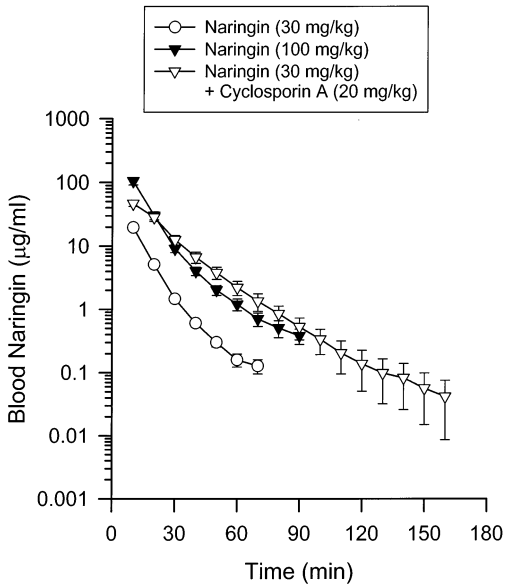

Figure 2. Mean naringin level in rat blood after naringin (30 and 100 mg/kg, i.v.) injection and with cyclosporin A (20 mg/kg, i.v.) co-administered. Data are presented as mean ± standard error of the mean (n = 6).

The amount of naringin, as estimated from the AUC, in bile against the concentration gradient was significantly greater than that in blood, suggesting that naringin might be excreted actively into the bile (**Figure 5**). Although these results indicate that the cyclosporin A-treated group showed increased naringin levels in all biological fluids, the distribution ratios of blood to liver (AUC_{liver}/AUC_{blood}) and blood to bile (AUC_{bile}/AUC_{blood}) were not affected (**Table 1**). The same tissues distribution phenomena have also been found at higher dosages of naringin

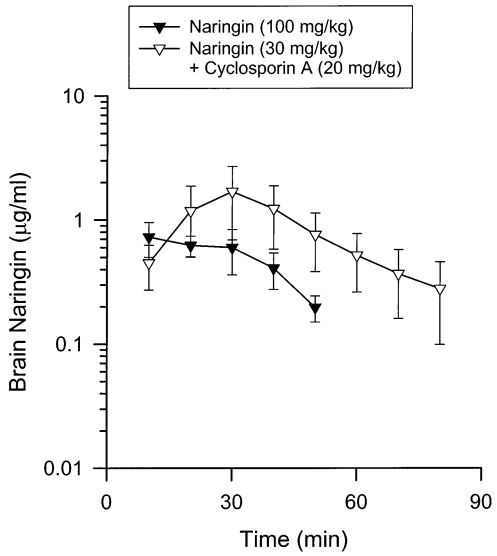

Figure 3. Mean naringin level in rat brain after naringin (100 mg/kg, i.v.) injection alone and naringin (30 mg/kg, i.v.) injection with cyclosporin A (20 mg/kg, i.v.) co-administered. Data are presented as mean ± standard error of the mean ($n = 6$).

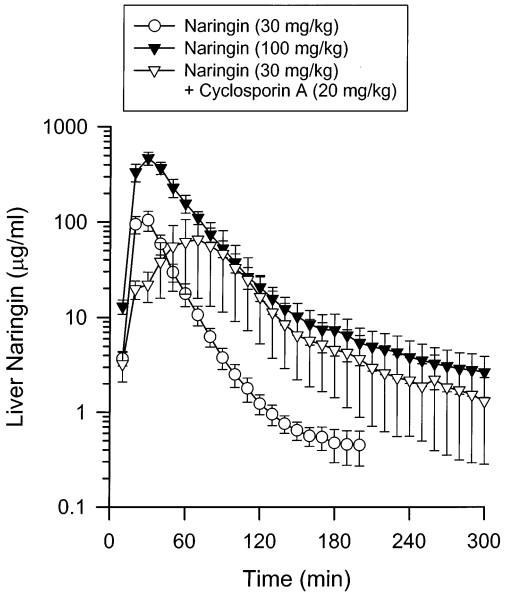

Figure 4. Mean naringin level in rat liver after naringin (30, 100 mg/kg, i.v.) injection and with cyclosporin A (20 mg/kg, i.v.) co-administered. Data are presented as mean ± standard error of the mean ($n = 6$).

(100 mg/kg) (**Table 2**). The results suggest that the concentration process of naringin has been carried out in the hepatobiliary excretion.

DISCUSSION

The results revealed the mechanism of the elimination pathway for hepatobiliary excretion of naringin. Microdialysis makes it possible to perform simultaneous and multiple sampling from biological fluids of smaller animals with anesthetized or freely moving status. Simultaneous multiple-site sampling in a single rat of blood, brain, liver, and bile has been developed in this study. Because there is no loss of biological fluids, microdialysis offers a number of advantages for pharmacokinetic studies, such as long sampling time and higher temporal resolution. A tissue homogenate technique has been utilized to

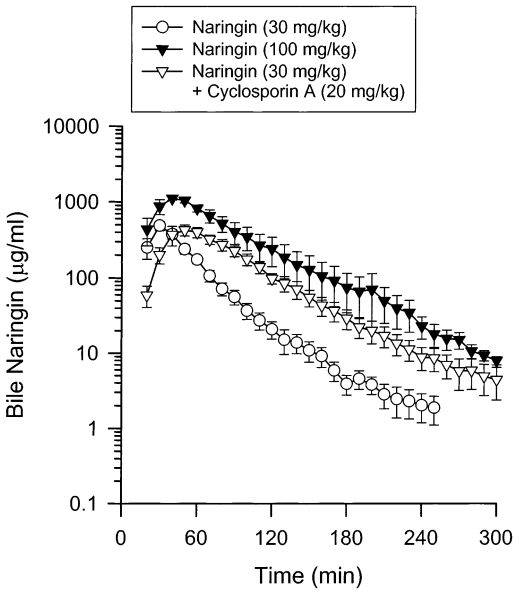

Figure 5. Mean naringin level in rat bile after naringin (30 and 100 mg/kg, i.v.) injection and with cyclosporin A (20 mg/kg, i.v.) co-administered. Data are presented as mean ± standard error of the mean ($n = 6$).

Table 1. Pharmacokinetic Data of Naringin (30 mg/kg) in Rat Blood, Brain, Liver, and Bile, Both with and without Cyclosporin A (20 mg/kg) Co-administered[a]

	naringin (30 mg/kg)	
drug treatment	without cyclosporin A	with cyclosporin A
	Blood	
AUC (min μg/mL)	667.96 ± 62.57	1450.82 ± 152.25[b]
	Brain	
T_{max} (min)	nd	20.00 ± 4.47
C_{max} (μg/mL)	nd	1.86 ± 0.96
AUC (min μg/mL)	nd	83.38 ± 40.03
	Liver	
T_{max} (min)	28.33 ± 1.67	46.67 ± 9.19
C_{max} (μg/mL)	108.11 ± 24.49	71.32 ± 48.53
AUC (min μg/mL)	3599.36 ± 626.55	5161.07 ± 3443.48
	Bile	
T_{max} (min)	31.67 ± 1.67	50.00 ± 3.65[b]
C_{max} (μg/mL)	488.28 ± 66.68	468.18 ± 85.61
AUC (min μg/mL)	19481.60 ± 2394.06	31715.60 ± 5836.99
AUC_{brain}/AUC_{blood}	nd	0.057 ± 0.027
AUC_{liver}/AUC_{blood}	5.39 ± 0.94	3.56 ± 2.37
AUC_{bile}/AUC_{blood}	29.17 ± 3.58	21.86 ± 4.02

[a] Data are expressed as mean ± standard error of the mean ($n = 6$). nd, Not detectable. [b] $P < 0.05$ significantly different from the group without cyclosporin A co-administered.

determine drug distribution in the brain. However, only single concentration−time points can be provided in such tissue homogenization studies, because the experimental animals need to be sacrificed to obtain the samples. Thus, to obtain a complete brain drug concentration−time profile, it is necessary to use many animals, and inter-animal variation often compromises the accuracy of the results. In particular, to study drug distribution from blood to brain, i.e., measuring the distribution ratio on the same animal, the drug concentration in the peripheral circulation and central nervous system should be measured simultaneously. Microdialysis has recently taken into account the major advanced sampling method with continuous, multiple sites and minimum tissue damage. These advantages of microdialysis have received the most attention and are widely used

Table 2. Pharmacokinetic Data of Naringin (100 mg/kg) in Rat Blood, Brain, Liver, and Bile[a]

parameter	estimate
Blood	
AUC (min μg/mL)	3507.31 ± 516.77
Brain	
T_{max} (min)	16.67 ± 3.33
C_{max} (μg/mL)	0.82 ± 0.22
AUC (min μg/mL)	23.78 ± 8.38
Liver	
T_{max} (min)	31.67 ± 1.67
C_{max} (μg/mL)	481.22 ± 72.59
AUC (min μg/mL)	20444.70 ± 2353.76
Bile	
T_{max} (min)	38.00 ± 4.90
C_{max} (μg/mL)	1162.51 ± 63.67
AUC (min μg/mL)	78258.00 ± 14355.60
AUC_{brain}/AUC_{blood}	0.0068 ± 0.0024
AUC_{liver}/AUC_{blood}	5.83 ± 0.67
AUC_{bile}/AUC_{blood}	22.31 ± 4.09

[a] Data are expressed as mean ± standard error of the mean ($n = 6$).

in neuroscience and pharmacokinetic applications to the brain (*25*). Naringin in brain dialysate could not be detected at the smaller dose of 30 mg/kg, but it was measured at the higher dose of 100 mg/kg. In the cyclosporin A concomitantly treated group, naringin in the brain has been detected at the smaller dose, suggesting that the influence of P-glycoprotein may be related to naringin crossing the BBB. This mechanism has confirmed P-glycoprotein on drug penetration of BBB (*26*).

Besides blood and brain sampling, a specially designed flow-through microdialysis probe provides a number of advantages for the hepatobiliary excretion (*19*) and enterohepatic circulation (*15*) study. This technique offers the advantage that the bile juice is not lost, and thus the physiological function of bile flow is not interrupted. Davies and Lunte (*27*) implanted three microdialysis probes into three different liver lobes. In this case, different metabolites have been identified due to regional differences in the levels of enzyme activity. Our research demonstrates that the sequential hepatobiliary excretion pathway of drug concentration from blood, liver, and bile can be simultaneously measured in the same rat. The naringin concentrations were gradually increasing from blood to bile, which was related to the transportation mechanism. The distribution ratios of AUC of liver and AUC of bile over that in blood of naringin were 5.39 ± 0.94 and 29.17 ± 3.58, respectively. However, the hepatobiliary excretion pathway of naringin does not diminish after cyclosporin A (a P-glycoprotein inhibitor) administration (**Table 1**).

Cyclosporin A is a cyclic undecapeptide whose larger structure involved in the numerous hydrophilic and hydrophobic compounds may be related to protein binding competition (*28*). Several reports suggest that grapefruit and its major ingredient, naringin, are involved in a drug−drug interaction (*29*, *30*). In this study, the naringin AUCs in blood were 667.96 ± 62.57 and 1450.82 ± 152.25 for the groups treated with naringin alone (30 mg/kg) and concomitantly treated with cyclosporin A, respectively (**Table 1**). Protein-unbound naringin concentration in blood was significantly enhanced with cyclosporin A co-administered. The concentration enhancement of naringin in blood may be related to the competition of protein binding resulting from the treatment with cyclosporin A. Not only the naringin in blood AUC was increased, but also those in both the liver and bile (**Table 1**). This concentration process of naringin in liver and bile may be due to the inhibition of hepatic enzyme activity and the P-glycoprotein transportation.

Naringin and its aglycon, naringenin, of the grapefruit flavonoid have been found in urinary excretion of healthy adults. The sugar moiety of naringin was removed by intestinal bacteria. These results suggest that the glucuronidation of naringenin and de-glucuronidation of naringin are the crucial steps in determining the bioavailability of the compound (*13*). In addition, our study provides evidence for the dominant pathway of hepatobiliary excretion of naringin, which goes through phase II glucuronide conjugation. Furthermore, the mechanism of this higher efficiency of hepatobiliary excretion of naringin may not be regulated by the P-glycoprotein.

In conclusion, we have developed a specific, rapid, and sensitive microbore HPLC method for the determination of protein-unbound naringin in rat blood, brain, liver, and bile. Current data obtained from rats show no significant impact of concomitantly injected cyclosporin A on the pharmacokinetics of naringin in rat blood, brain, liver, and bile.

ACKNOWLEDGMENT

The author thanks Ms. Yen-Fei Chen of the National Research Institute of Chinese Medicine for her technical assistance.

LITERATURE CITED

(1) Ueng, Y. F.; Chang, Y. L.; Oda, Y.; Park, S. S.; Liao, J. F.; Lin, M. F.; Chen, C. F. In vitro and in vivo effects of naringin on cytochrome P450-dependent monooxygenase in mouse liver. *Life Sci.* **1999**, *65*, 2591−2602.

(2) Ameer, B.; Weintraus, R. A. Drug interactions with grapefruit juice. *Clin. Pharmacokinet.* **1997**, *31*, 103−121.

(3) Davies, M. I. A review of microdialysis sampling for pharmacokinetic applications. *Anal. Chim. Acta* **1999**, *379*, 227−249.

(4) De Lange, E. C. M.; Danhof, M.; De Boer, A. G.; Breimer, D. D. Methodological considerations of intracerebral microdialysis in pharmacokinetic studies on drug transport across the blood-brain barrier. *Brain Res. Rev.* **1997**, *25*, 27−49.

(5) Tossman, U.; Ungerstedt, U. Microdialysis in the study of extracellular levels of amino acids in the rat brain. *Acta Physiol. Scand.* **1986**, *128*, 9−14.

(6) Zetterstrom, T.; Sharp, T.; Marsden, C. A.; Ungerstedt, U. *In vivo* measurement of dopamine and its metabolites by intracerebral dialysis: changes after d-amphetamine. *J. Neurochem.* **1983**, *41*, 1769−1773.

(7) Stenken, J. A.; Lunte, C. E.; Southard, M. Z.; Stahle, L. Factors that influence microdialysis recovery. Comparison of experimental and theoretical microdialysis recoveries in rat liver. *J. Pharm. Sci.* **1997**, *86*, 958−966.

(8) Johansen, M. J.; Newman, R. A.; Madden, T. The use of microdialysis in pharmacokinetics and pharmacodynamics. *Pharmacotherapy* **1997**, *17*, 464−481.

(9) Scott, D. O.; Sorenson, L. R.; Steele, K. L.; Puckett, D. L.; Lunte, C. E. *In vivo* microdialysis sampling for pharmacokinetic investigations. *Pharm. Res.* **1991**, *8*, 389−392.

(10) Stahle, L. In *Techniques in the Behavioral and Neural Science*; Robinson, T. E., Justice, J. B., Eds.; Elsevier: Amsterdam, 1991; Vol. 7, pp 155−174.

(11) Fuhr, U.; Klittich, K.; Staib, A. H. Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man. *Br. J. Clin. Pharmacol.* **1993**, *35*, 431−436.

(12) Scambia, G.; Ranelletti, F. O.; Panici, P. B.; De Vincenzo, R.; Bonanno, G.; Ferrandina, G.; Piantelli, M.; Bussa, S.; Rumi, C.; Cianfriglia, M. Quercetinpotentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast-cancer cell line: P-glycoprotein as a possible target. *Cancer Chemother. Pharmacol.* **1994**, *34*, 459−464.

(13) Fuhr, U.; Kummert, A. L. The fate of naringin in humans: a key to grapefruit juice-drug interactions? *Clin. Pharmacol. Ther.* **1995**, *58*, 365−373.
(14) Tsai, T. H.; Chen, Y. F.; Chen, I. F.; Chen, C. F. Measurement of unbound caffeic acid in rat blood by on-line microdialysis coupled with liquid chromatography and its application to pharmacokinetic studies. *J. Chromatogr. B* **1999**, *729*, 119−125.
(15) Tsai, T. H.; Shum, A. Y. C.; Chen, C. F. Enterohepatic circulation of chloramphenicol and its glucuronide in the rat by microdialysis using a hepato-duodenal shunt. *Life Sci.* **2000**, *66*, 363−370.
(16) Scott, D. O.; Lunte, C. E. *In vivo* microdialysis sampling in the bile, blood, and liver of rats to study the disposition of phenol. *Pharm. Res.* **1993**, *10*, 335−342.
(17) Tsai, T. H.; Huang, C. T.; Shum, A. Y. C.; Chen, C. F. Pharmacokinetic and biliary excretion study of esculetin by microdialysis in rat. *Life Sci.* **1996**, *65*, 1647−1655.
(18) Tsai, T. H.; Hung, L. C.; Chen, C. F. Biliary excretion of chloramphenicol and chloramphenicol glucuronide in rat by microdialysis. *J. Pharm. Pharmacol.* **1999**, *51*, 911−915.
(19) Tsai, T. H. Pharmacokinetics of pefloxacin and its interaction with cyclosporin A, a P-glycoprotein modulator, in rat blood, brain and bile, using simultaneous microdialysis. *Br. J. Pharmacol*. **2001**, *132*, 1310−1316.
(20) Tsai, T. H.; Lee, C. H.; Yeh, P. H. Effect of cyclosporin A, a P-glycoprotein modulator, on the disposition and biliary excretion of camptothecin using microdialysis. *Br. J. Pharmacol*. **2001**, *134*, 1245−1252.
(21) Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*; Academic Press: San Diego, CA, 1986.
(22) Tsai, T. H.; Chen, C. F. Simultaneous measurement of acetylcholine and monoamines by two serial on-line microdialysis systems: effects of methamphetamine on neurotransmitters release from the striatum of freely moving rats. *Neurosci. Lett*. **1994**, *166*, 175−177.
(23) Gabrielsson, J.; Weiner, D. Non-compartmental analysis. In *Pharmacokinetic and Pharmacodynamic Data Analysis Concepts and Applications*; Swedish Pharm. Press: Stockholm, 1994; pp 621−626.
(24) Abanmi, N.; Zaghloul, I.; El Sayed, N.; Al-Khamis, K. I. Determination of naringin and its main active metabolite in human serum by high-performance liquid chromatography. *Ther. Drug Monitor.* **1996**, *31*, 158−163.
(25) Hammarlund-Udenaes, M. The use of microdialysis in CNS drug delivery studies: pharmacokinetic perspectives and results with analgesics and antiepileptics. *Adv. Drug Del. Rev.* **2000**, *45*, 283−294.
(26) Schinkel, A. H.; Wagenaar, E.; Mol, C. A. A. M.; Van Deemter, L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological and activity of many drugs. *J. Clin. Invest.* **1996**, *97*, 2517−2524.
(27) Davies, M. I.; Lunte, C. E. Simultaneous microdialysis sampling from multiple sites in the liver for the study of phenol metabolism. *Life Sci.* **1996**, *59*, 1001−1013.
(28) Kubo, M.; Kincaid, R. L.; Webb, D. R.; Ransom, J. T. The Ca^{2+}/calmodulin-activated, phosphoprotein phosphatase calcineurin is sufficient for positive transcriptional regulation of the mouse IL-4 gene. *Int. Immunol.* **1994**, *6*, 179−188.
(29) Fuhr, U.; Maier-Bruggemann, A.; Blume, H.; Muck, W.; Unger, S.; Kuhlmann, J.; Huschka, C.; Zaigler, M.; Rietbrock, S.; Staib, A. H. Grapefruit juice increases oral nimodipine bioavailability. *Int. J. Clin. Pharmacol. Ther.* **1998**, *36*, 126−132.
(30) Zhang, H.; Wong, C. W.; Coville, P. F.; Wanwimolruk, S. Effect of the grapefruit flavonoid naringin on pharmacokinetics of quinine in rats. *Drug Metab. Drug Interact.* **2000**, *17*, 351−363.

Received for review May 28, 2002. Revised manuscript received August 22, 2002. Accepted August 24, 2002. This study was supported in part by research grants (NSC91-2113-M-077-008; NSC91-2320-B-077-012) from the National Science Council, Taiwan.

JF020603P