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The effects of the cyclosporin A, a P-glycoprotein inhibitor, on the pharmacokinetics of baicalein in the rat: a microdialysis study

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1 Baicalein is a bioactive flavonoid isolated from the root of *Scutellaria baicalensis* Georgi, a medicinal herb that has been used since ancient times to treat bacterial infections. As little is known concerning its pharmacokinetics, this study focussed on its pharmacokinetics as well as the possible roles of the multidrug transporter P-glycoprotein on its distribution and disposition.

2 Three microdialysis probes were simultaneously inserted into the jugular vein, the hippocampus and the bile duct of male Sprague–Dawley rats for sampling in biological fluids following the administration of baicalein (10, 30 and 60 mg kg^{-1}) through the femoral vein. The P-glycoprotein inhibitor cyclosporin A was used to help delineate its roles.

3 The study design consisted of two groups of six rats in parallel: control rats which received baicalein alone and the cyclosporin A treated-group in which the rats were injected cyclosporin A, a P-glycoprotein inhibitor, 10 min prior to baicalein administration.

4 Cyclosporin A treatment resulted in a significant increase in elimination half-life, mean residence time and area under the concentration versus time curve (AUC) of unbound baicalein in the brain. However, AUC in the bile was decreased.

5 The decline of baicalein in the hippocampus, blood and bile suggested that there was rapid exchange and equilibration between the peripheral compartment and the central nervous system. In addition, the results indicated that baicalein was able to penetrate the blood–brain barrier as well as undergoing hepatobiliary excretion.

6 Although no direct transport studies were undertaken and multiple factors may affect BBB penetration and hepatobiliary excretion, strong association of the involvement of P-glycoprotein in these processes is indicated.

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Abbreviations: AUC, area under the concentration *vs* time curve; AUMC, area under the first moment curve; BBB, blood–brain barrier; Cl, clearance; C_{max}, peak concentration; CSF, cerebrospinal fluid; MDR, multidrug resistance; MRT, mean residence time; RBC, red blood cell; $t_{\frac{1}{2}}$, elimination half-life.

Introduction

Scutellaria baicalensis Georgi is a traditional herbal medicine widely used in traditional herbal preparations in Taiwan, Japan and China. Baicalein (Figure 1) is a flavonoid isolated from the root of *Scutellaria baicalensis* Georgi whose pharmacological properties have been reported to include anti-inflammatory actions (Lin & Shieh, 1996), inhibition of leukotrienes B_4 and C_4 syntheses, degranulation of human polymorphonuclear leukocytes (Kimura *et al*., 1986), attenuation of lipid peroxidation in rat liver (Kimura *et al*., 1984) and stimulation of adhesion molecule expressions induced by inflammatory cytokines such as IL-1α and TNF-α (Kimura *et al*., 1997). However, relatively little is known concerning its pharmacokinetics. As the herb is widely used and its active component baicalein possessing potentially multiple actions, it is of interest to study its pharmacokinetics.

According to classical pharmacokinetics, once a drug gets into the bloodstream, it must be distributed to the site(s) of action in order to exert its own effects. Many drugs bind to plasma proteins such as albumin (mainly anionic compounds) and alpha 1-acid glycoprotein (cationic compounds) (Meijer & van der Sluijs, 1989). However, only the protein-unbound drugs are available for redistribution or activation of responses. Traditional biological fluids sampling method represent measurements of total drug concentrations, which do not necessarily reflect the intensities of the pharmacological responses. Microdialysis, which samples only small molecular substances, is uniquely suitable for continuous monitoring of protein-unbound drugs in biological fluids as the larger protein-bound molecules are excluded, the technique itself is relatively non-invasive and non-consumptive (Weiss *et al*., 2000). In the present study, simultaneous insertion of microdialysis probe in the jugular vein, the hippocampus and the bile duct permitted the simultaneous monitoring of the level of protein-unbound drug fractions in various compartments and provided information concerning the distribution in different compartments and transportation across biological barriers of the drug of interest which in our case was baicalein.

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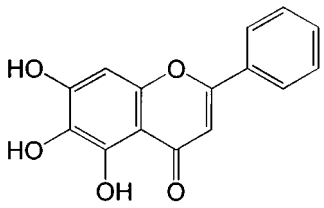

Figure 1 Chemical structure of baicalein.

The blood–brain barrier (BBB) provides a formidable barrier between the blood and parenchyma of the brain as substances need to traverse the endothelial cells lining brain capillaries. However, various transport systems help to facilitate transportation of select substances across the BBB. The nature of such transportation has been intensely studied. Since the finding of the multidrug transporter P-glycoprotein in normal brain capillary endothelial cells (Thiebaut *et al*., 1989) and the functional expression as a drug efflux transporter at BBB (Jolliet-Riant & Tillement, 1999), its role in regulating the movements of substances across the BBB has been a subject of intense interest. P-glycoprotein has also been implicated in the regulation of liver function (Fardel *et al*., 1992; Ziemann *et al*., 1999) and hepatobiliary transport, a major system by which endogenous and exogenous compounds are excreted (Schinkel, 1997; Gupta *et al*., 2000) and is mediated by the coordinated action of multiple transport systems present at the sinusoidal and canalicular membrane domains of hepatocytes (Hooiveld *et al*., 2000). Although the physiologic function of P-glycoprotein is not fully understood (Johnstone *et al*., 2000), it has been shown to be present in the liver canalicular membrane and to play important roles in different pharmacokinetic steps (Leveque & Jehl, 1995). Recent reports indicate that drug-transporting P-glycoproteins are abundant in the liver and the intestinal wall (Hebert, 1997; Meijer *et al*., 1997). In the rat, the carcinogens 2-acetylaminofluorene-induced *mdr* (multidrug resistance) gene expression leads to increased vinblastine excretion into the bile (Schrenk *et al*., 1993). Thus, SDZ PSC-833, a P-glycoprotein inhibitor, significantly decreased the hepatobiliary excretion of colchicine and doxorubicin (Speeg & Maldonado, 1994).

Hence, our hypothesis was that baicalein might be actively transported across the sinusoidal membrane into hepatocyte, and secreted into biliary canaliculi *via* P-glycoprotein. The aim of this study was to characterize the pharmacokinetics of herbal ingredient baicalein and its interaction with P-glycoprotein. The results indicated that cyclosporin A treatment had significant effects on the pharmacokinetics of baicalein, facilitating BBB penetration while suppressing biliary excretion. Although we did not attempt transport studies directly linked to P-glycoprotein and the effects of cyclosporin A and that multiple factors may affect the availability of unbound drugs which formed the basis of our pharmacokinetic computations and cyclosporin A itself may have additional effects other than P-glycoprotein inhibition, the reputed presence of P-glycoprotein in these tissues and inhibitory effects of cyclosporin A suggest strongly the involvement of P-glycoprotein in these processes.

Methods

Experimental animals

All experimental protocols involving animals had been reviewed and approved by the institutional animal experimentation committee of the National Research Institute of Chinese Medicine. Male specific pathogen-free Sprague–Dawley rats weighing 250–300 g were obtained from the Laboratory Animal Center of the National Yang-Ming University, Taipei, Taiwan. Following arrival at the animal facilities, there was a minimum of 7 days of acclimatization during which they had free access to food (Laboratory Rodent Diet no. 5P14, PMI Feeds Inc., Richmond, IN, U.S.A.) and water until 18 h prior to being used in experiments, at which time only food was removed. The rats were initially anaesthetized with urethane 1 g ml^{-1} and α-chloralose 0.1 g ml^{-1} (1 ml kg^{-1}, i.p.), and remained anaesthetized throughout the experimental period. The femoral vein was exposed for further drug administration. The rat's body temperature was maintained at 37°C with a heating pad.

Chemicals and reagents

Baicalein was purchased from Aldrich Chem. Co. (Milwaukee, WI, U.S.A.). Cyclosporin A (Sandimmun) was obtained from Novartis Pharma (Basle, Switzerland). Chromatographic grade solvents were purchased from BDH (Poole, U.K.). Triply de-ionized water from Millipore (Bedford, MA, U.S.A.) was used for all preparations.

Chromatography

Liquid chromatographic grade solvents and reagents were obtained from E Merck (Darmstadt, Germany). Triply deionized water (Millipore, Bedford, MA, U.S.A.) was used for all preparations. The HPLC system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN, U.S.A.), an off-line fraction collector (CMA 140, Stockholm, Sweden) equipped with a 10 μl sample loop, plus an electrochemical detector (BAS 4C). Resolution was achieved using a microbore reversed phase C-18 column (150 × 1 mm I.D.; particle size 5 μm). The mobile phase was comprised of 400 ml acetonitrile, 8 ml methanol, 0.1 M NaH_2PO_4, 0.5 mM 1-octanesulphonate and 0.1 mM EDTA in 1 l of doubly-distilled water. The solution was adjusted to pH 4.5 with orthorphosphoric acid (85%) and the flow rate of the mobile phase was 0.05 ml min^{-1}. The mobile phase was filtered through a Millipore 0.22 μm filter and degassed prior to use. Detection was achieved electrochemically coupling a glassy carbon working electrode to a reference Ag/AgCl electrode with applied potential set at 0.5 V. Output data from the detector were integrated *via* an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, U.S.A.).

Microdialysis experiment

Blood, brain and bile microdialysis systems consisted of a microinjection pump (CMA/100, Stockholm, Sweden), a fraction collector (CMA/140) and microdialysis probes. The dialysis probes for blood (1 cm in the length for dialysis) and brain (0.3 cm in the length for dialysis) (Tsai *et al*., 1999a)

were made of silica capillary in a concentric design. The tips of microdialysis probes were covered by dialysis membranes (Spectrum, 150 μm outer diameter with a cut-off at nominal molecular mass of 13,000, Laguna Hills, CA, U.S.A.). The blood microdialysis probe was positioned within the jugular vein/right atrium (toward the heart) and then perfused with anticoagulant citrate dextrose, ACD solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM) at a flow-rate of 1 μl min^{-1}.

The bile duct microdialysis probes were constructed in our own laboratory (Tsai *et al*., 1999b; Tsai, 2001) according to the design originally described by Scott & Lunte (1993) and Hadwiger *et al*. (1994). A 7 cm dialysis membrane (Spectrum, 150 μm outer diameter with a cut-off at nominal molecular mass of 13,000) was inserted into a polyethylene tubing (PE-60; 0.76 mm i.d.; 1.22 mm o.d., Clay-Adams, N.J., U.S.A.). The ends of the dialysis membrane and PE-60 were inserted into a silica tubing (40 μm i.d; 140 μm o.d., SGE, Australia) and PE-10 (0.28 mm i.d.; 0.61 mm o.d.), respectively. Both the ends of tubing and the union were cemented with epoxy. At least 24 h were allowed for the epoxy to dry. After bile duct cannulation, the probe was then perfused with Ringer's solution at a flow rate of 2.6 μl min^{-1}.

After the implantation of the blood and bile microdialysis probes, the rat was immobilized in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, U.S.A.). The skull was surgically exposed, and a hole was trephined into the skull based on stereotaxic coordinates (Paxinos & Watson, 1982). The brain microdialysis probe was placed into the right hippocampus (5.6 mm posterior to bregma, 5.0 mm lateral to midline and 7.0 mm lower to tip). The brain microdialysis probe was perfused with Ringer's solution (147 mM Na^+; 2.2 mM Ca^{2+}; 4 mM K^+; pH 7.0) at a flow-rate of 1 μl min^{-1}. Brain dialysates were collected by a fraction collector (CMA/140) at 10 min intervals. The position of each brain microdialysis probe was verified at the end of the experiments (Tsai & Chen, 1994; Huang *et al*., 1999).

The *in vivo* probe recovery was determined by estimating the loss (the extraction ratio) of the baicalein, which was calculated from the concentration in the dialysate (C_{out}) relative to the concentration of the baicalein in the perfusate (C_{in}). Recovery (R_{dial}) was expressed by the following equation: $R_{dial} = 1 - (C_{out}/C_{in})$. The *in vivo* recovery of microdialysates for rat blood (Tsai *et al*., 1999a), brain (Huang *et al*., 1999) and bile (Tsai *et al*., 1999b; Tsai, 2001) using microdialysis probes have been described in previous reports. All dialysates were analysed by the HPLC system modified from that reported by Wakui *et al*. (1992).

Drug treatment

After a 2-h post-surgical stabilization period, baicalein (10, 30 or 60 mg kg^{-1}, i.v.) was administered in the control group ($n=6$). For cyclosporin A treated groups, 20 mg kg^{-1} of cyclosporin A was injected *via* the left femoral vein 10 min prior to baicalein injection, respectively. The volume of each injection was 1 ml kg^{-1}.

Pharmacokinetic analysis

Baicalein concentrations in blood, brain and bile were corrected by the estimated *in vivo* recoveries from the respective microdialysis probes. The midpoint of the 10 min periods was used as the sample time for blood, brain and bile baicalein microdialysate concentration-time profiles. Baicalein microdialysate concentrations (C_m) were converted to unbound concentration (C_u) as follows: $C_u = C_m/R_{dial}$ (Evrard *et al*., 1996). 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, U.S.A.) by the noncompartmental method (Benet & Galeazzi, 1979; Gabrielsson & Weiner, 1994).

Statistics

The results are represented as mean $\pm$ standard error of the mean. Statistical analysis was performed by Student's *t*-test (SPSS version 10.0, Chicago, IL, U.S.A.) for comparisons between the control (baicalein treated alone) and P-glycoprotein treated groups. The level of significance was set at $P<0.05$.

Results

Method validation

Liquid chromatographic resolution coupled to electrochemical detection was used for the determination of baicalein in dialysates of blood, brain tissue and bile following drug administration. The intra-day and inter-day precision and accuracy of baicalein fell well within the predefined limits of acceptability and was sensitive enough for the present purposes. The average microdialysate recoveries of baicalein for blood, brain, and bile were 0.40, 0.22, and 0.73, respectively.

Brain distribution of baicalein

The brain tissue concentration of baicalein revealed no significant variations among various regions (brain stem, cerebellum, cerebral cortex, hippocampus, midbrain, and striatum) after 20 min of baicalein administration (60 mg kg^{-1}, i.v.). The method has been described in a previous report (Tsai *et al*., 2001). The cerebrospinal fluid concentration of baicalein was about 10–20% of brain regional concentration. The mean dialysate concentration of baicalein in each brain region was approximately one third of that in plasma. The distribution of baicalein into red blood cell cytoplasm was about 12% of its plasma concentration (Table 1).

Dose dependence of baicalein in rat blood

Mean unbound baicalein blood concentrations versus time profiles for doses administered (10, 30 and 60 mg kg^{-1}, i.v.) are presented in Figures 2–4. The pharmacokinetic parameters, as derived from these data and calculated by the pharmacokinetic program (WinNonlin), are shown in Tables 2–4. The pharmacokinetic profiles suggest that the AUC of baicalein in rat blood appeared in a dose-dependent manner and the clearance (Cl), elimination half-life ($t_{\frac{1}{2}}$), and mean residence time (MRT) were not significantly different showing

Table 1 Mean baicalein concentration in rat brain regions ($\mu g\ g^{-1}$) and plasma ($\mu g\ ml^{-1}$) at 20 min after baicalein administration (60 mg kg^{-1}, i.v.)

Brain regions and plasma	*Concentrations*
Brain regions	
Brainstem	4.05±0.57
Cerebellum	3.13±0.39
Cerebral cortex	2.72±0.51
Hippocampus	3.17±0.46
Midbrain	4.09±1.09
Striatum	3.00±0.56
CSF	0.21±0.031*
Blood regions	
RBC cytoplasm	1.22±0.40
Plasma	9.81±1.28*

Data are expressed as mean±s.e.mean. ($n=4$). *$P<0.05$ CSF and plasma concentration compare with each of brain and blood regions, respectively.

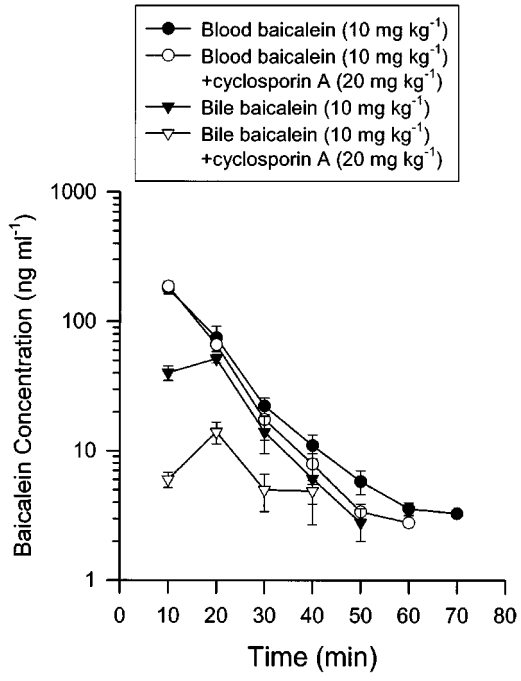

Figure 2 Concentration-time profiles for baicalein in blood and bile after baicalein i.v. administration at dosage of 10 mg kg^{-1} with and without cyclosporin A (20 mg kg^{-1}) administration. The data are means±s.e.mean from six individual microdialysis experiments.

linear phenomenon at the dose range of 10 to 60 mg kg^{-1}. The elimination half-lives of baicalein from the brain compartment was about twice as long as that observed in the blood compartment at doses of 30 and 60 mg kg^{-1} (Tables 3 and 4).

Baicalein pharmacokinetics in blood and bile

Mean unbound baicalein bile concentrations versus time profiles for the dosages of 10, 30 and 60 mg kg^{-1} are presented in Figures 2–4. The concentration of baicalein in bile gradually increased and reached peak concentration in about 20–30 min. The hepatobiliary excretion of baicalein,

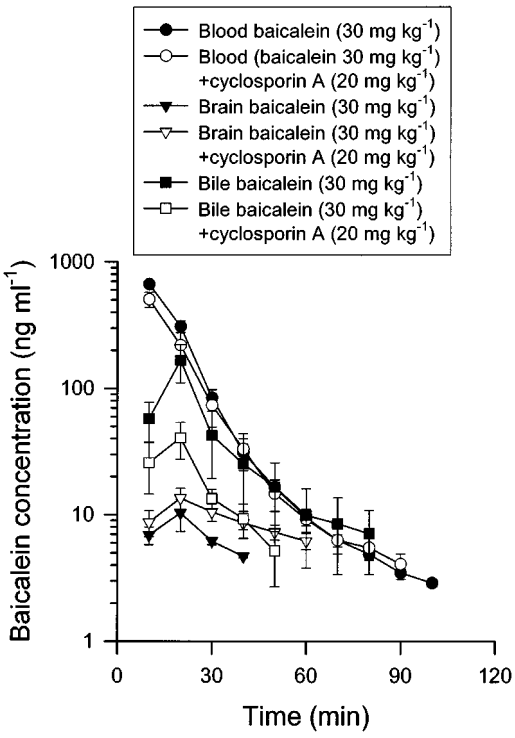

Figure 3 Concentration-time profiles for baicalein in blood, brain, and bile after baicalein i.v. administration at dosage of 30 mg kg^{-1} with and without cyclosporin A (20 mg kg^{-1}) administration. The data are means±s.e.mean from six individual microdialysis experiments.

defined as the blood-to-bile distribution (k value), was calculated by dividing the baicalein AUC in bile by that in blood ($k=AUC_{bile}/AUC_{blood}$) (De Lange *et al*., 1997). The blood-to-bile distribution ratios were 0.21±0.012, 0.18±0.040, and 0.18±0.009 after baicalein injection at doses of 10, 30 and 60 mg kg^{-1}, respectively.

Cyclosporin A and baicalein interaction in rat blood

Concomitant administration of cyclosporin A did not result in significant alterations in blood baicalein concentrations (Figures 2–4). The blood AUC's of baicalein both alone and in the cyclosporin A treated group were 5477±493, 18859±1063, 39094±8863 min ng ml^{-1}, and 5930±1084, 16656±2484, 33311±3729 min ng ml^{-1}, respectively at doses of 10, 30, and 60 mg kg^{-1} baicalein administered (Tables 2–4). The results suggest that cyclosporin A did not alter the pharmacokinetics of baicalein in the blood.

Cyclosporin A and baicalein interaction in rat bile

Coadministration of cyclosporin A with baicalein at doses of 10, 30, and 60 mg kg^{-1} resulted in the baicalein levels in the bile being dramatically decreased (Figures 2–4). The bile AUC's of baicalein (10, 30, and 60 mg kg^{-1}) alone and with cyclosporin A were 1145±115, 3335±892, 7027±1170, and 459±102, 1045±184, 2601±569 min ng ml^{-1}, respectively. Similarly, the peak concentrations (C_{max}) of baicalein in rat bile were significantly reduced with cyclosporin A treatment. The significant reduction in the blood-to-bile distribution at

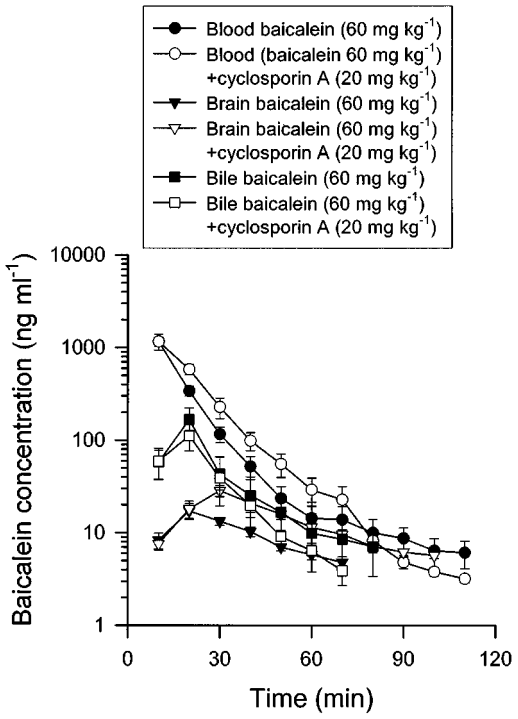

Figure 4 Concentration-time profiles for baicalein in blood, brain, and bile after baicalein i.v. administration at dosage of 60 mg kg^{-1} with and without cyclosporin A (20 mg kg^{-1}) administration. The data are means ± s.e.mean from six individual microdialysis experiments.

Table 2 Pharmacokinetic data of baicalein (10 mg kg^{-1}) in rat blood, brain and bile, with and without cyclosporin A (20 mg kg^{-1})

	Baicalein (10 mg kg^{-1})	
Drug treatment	*Without cyclosporin A*	*With cyclosporin A*
Blood		
AUC (min ng ml^{-1})	5477 ± 493	5930 ± 1084
Cl (1 kg^{-1} min^{-1})	1.9 ± 0.2	1.9 ± 0.3
$t_{\frac{1}{2}}$ (min)	9.9 ± 1.0	8.4 ± 1.1
MRT (min)	9.8 ± 1.0	8.2 ± 1.3
Brain		
AUC (min ng ml^{-1})	n.d.	n.d.
Cl (1 kg^{-1} min^{-1})	n.d.	n.d.
$t_{\frac{1}{2}}$ (min)	n.d.	n.d.
MRT (min)	n.d.	n.d.
Bile		
AUC (min ng ml^{-1})	1145 ± 115	459 ± 1.02*
Cl (1 kg^{-1} min^{-1})	53.8 ± 3.8	14.0 ± 2.7*
$t_{\frac{1}{2}}$ (min)	5.8 ± 0.8	34.0 ± 14.2*
MRT (min)	19.2 ± 1.5	52.0 ± 18.7*
AUC_{bile}/AUC_{blood}	0.21 ± 0.012	0.079 ± 0.008*

Data are expressed as mean ± s.e.mean. ($n=6$). *$P<0.05$ Significantly different from the control group. n.d.: levels are not detectable.

all three doses (10, 30, and 60 mg kg^{-1}) (Tables 2–4) suggests that the bile efflux transport system of baicalein might be inhibited by the treatment of cyclosporin A.

Table 3 Pharmacokinetic data of baicalein (30 mg kg^{-1}) in rat blood, brain and bile, with and without cyclosporin A (20 mg kg^{-1})

	Baicalein (30 mg kg^{-1})	
Drug treatment	*Without cyclosporin A*	*With cyclosporin A*
Blood		
AUC (min ng ml^{-1})	18859 ± 1063	16656 ± 2484
Cl (1 kg^{-1} min^{-1})	1.6 ± 0.1	1.9 ± 0.3
$t_{\frac{1}{2}}$ (min)	11.7 ± 0.4	10.7 ± 1.9
MRT (min)	10.4 ± 0.9	11.3 ± 1.8
Brain		
AUC (min ng ml^{-1})	436 ± 28	921 ± 175*
C_{max} (ng ml^{-1})	10.4 ± 2.9	13.5 ± 2.6
$t_{\frac{1}{2}}$ (min)	26.4 ± 3.8	43.2 ± 6.4*
MRT (min)	45.8 ± 5.8	70.8 ± 8.8*
Bile		
AUC (min ng ml^{-1})	3335 ± 892	1045 ± 184*
C_{max} (ng ml^{-1})	171.1 ± 55.4	51.0 ± 12.1*
$t_{\frac{1}{2}}$ (min)	11.8 ± 3.0	13.7 ± 3.5
MRT (min)	25.3 ± 3.5	26.9 ± 4.2
AUC_{brain}/AUC_{blood}	0.023 ± 0.001	0.055 ± 0.004*
AUC_{bile}/AUC_{blood}	0.18 ± 0.04	0.065 ± 0.006*

Data are expressed as mean ± s.e.mean. ($n=6$). *$P<0.05$ Significantly different from the control group.

Table 4 Pharmacokinetic data of baicalein (60 mg kg^{-1}) in rat blood, brain and bile, with and without cyclosporin A (20 mg kg^{-1})

	Baicalein (60 mg kg^{-1})	
Drug treatment	*Without cyclosporin A*	*With cyclosporin A*
Blood		
AUC (min ng ml^{-1})	39094 ± 8863	33311 ± 3729
Cl (1 kg^{-1} min^{-1})	1.9 ± 0.4	1.9 ± 0.3
$t_{\frac{1}{2}}$ (min)	12.3 ± 2.3	11.8 ± 0.4
MRT (min)	8.8 ± 1.8	12.4 ± 1.2
Brain		
AUC (min ng ml^{-1})	839 ± 83	1638 ± 170*
C_{max} (ng ml^{-1})	18.0 ± 2.7	28.5 ± 3.9*
$t_{\frac{1}{2}}$ (min)	29.0 ± 4.3	49.6 ± 10.7*
MRT (min)	52.4 ± 6.3	75.6 ± 9.3*
Bile		
AUC (min ng ml^{-1})	7027 ± 1170	2601 ± 569*
C_{max} (ng ml^{-1})	307.8 ± 49.5	118.7 ± 35.3*
$t_{\frac{1}{2}}$ (min)	8.4 ± 1.6	27.7 ± 13.9
MRT (min)	23.5 ± 2.9	30.9 ± 5.0
AUC_{brain}/AUC_{blood}	0.023 ± 0.003	0.049 ± 0.002*
AUC_{bile}/AUC_{blood}	0.18 ± 0.009	0.079 ± 0.009*

Data are expressed as mean ± s.e.mean. ($n=6$). *$P<0.05$ Significantly different from the control group.

Cyclosporin A and baicalein interaction in rat brain

Brain baicalein was not detectable at the low dose of 10 mg kg^{-1} but were detected at the higher doses of 30 and 60 mg kg^{-1}, indicating that at these levels baicalein was able to penetrate the blood–brain barrier. Coadministration of cyclosporin A significantly increased the brain level of baicalein (Figures 3 and 4). The brain AUC's of baicalein

(30, and 60 mg kg^{-1}) alone and with cyclosporin A were 436 ± 28, 839 ± 83, and 921 ± 175, 1638 ± 170 min ng ml^{-1}, respectively. The peak concentration (C_{max}) of baicalein at the highest dose of 60 mg kg^{-1} was significantly higher with the treatment of cyclosporin A, going from 18.0 ± 2.7 to 28.5 ± 3.9 ng ml^{-1} (Table 4). The blood-to-brain distribution was significantly increased when cyclosporin A was co-administered with the doses of baicalein used (30, and 60 mg kg^{-1}) (Tables 3 and 4), which suggested that the brain transport system of baicalein might be regulated by P-glycoprotein.

Discussion

Excluding large molecules such as protein bound molecules and being relatively non-invasive, non-perturbing and non-consumptive, microdialysis is particularly suitable for continuous monitoring of the disposition of small molecules and therefore the pharmacokinetics and in particular that of the protein unbound fraction. In this study we employed multiple probe placements in blood, brain and bile for simultaneous sampling in different compartments representing the peripheral and central compartments as well as a major metabolic-excretory route. Scott & Lunte (1993) used *in vivo* microdialysis technique to investigate the hepatic metabolism and hepatobiliary excretion of phenol in rat blood, liver and bile.

Urethane was employed as the anaesthetic in our study due to its reported long action (Waynforth & Flecknell, 1992). Urethane also provides a stable heart rate and blood pressure in deep anaesthetic stage maintained for approximately 4 h during the experiment. Maintaining deep anaesthesia is required for studies with multiple probes microdialysis sampling.

Baicalein appeared to be homogeneously distributed over a wide range of brain areas. Although it is evenly distributed in various regions of the brain, we chose the hippocampus as the site for sampling as baicalein has been reported to possess free radical scavenging actions and the hippocampus is an important area particularly vulnerable to damage by free radicals (Hamada *et al*., 1993).

Several theories have been proposed to explain the interaction between flavonoid and cyclosporin A. These include the displacement of flavonoid from plasma proteins, competition between flavonoid and cyclosporin A for renal tubular excretion, reduction in flavonoid clearance secondary to renal capillary constriction induced by cyclosporin A, and impaired hepatic metabolism of flavonoid (Di *et al*., 2002). The present data support both displacement and reduced biliary elimination of baicalein. However, displacement reactions are not of a magnitude to be of potential clinical importance unless the compound to be displaced is more than 90% protein bound and has an apparent volume of distribution not exceeding the volume of extracellular water. Thus, it seems more likely that a potential displacement reaction involving the metabolite of baicalein is of greater importance than any displacement involving the parent compound.

The blood-to-bile distribution ratios (AUC_{bile}/AUC_{blood}) of baicalein were 0.21 ± 0.012, 0.18 ± 0.04, and 0.18 ± 0.009, following baicalein 10, 30, and 60 mg kg^{-1} administration, respectively. The relatively low biliary distribution ratios suggest that the major product of hepatobiliary of baicalein was not the parent compound itself but the metabolites. Our results supported the findings of Abe *et al*. (1990) that the major metabolites of baicalein in rat bile underwent phase two conjugation.

At the dose of 20 mg kg^{-1} of cyclosporin A treatment, the hepatobiliary excretion of baicalein was reduced significantly ($P < 0.001$) in all three doses (10, 30 and 60 mg kg^{-1}). However, the blood AUC of baicalein in among doses were not significantly changed in the cyclosporin A treated groups. Many studies have shown that P-glycoprotein may play a transportation role in excreting some drugs from the liver to the bile (Meijer *et al*., 1997). ATPase transporter proteins are commonly found in the hepatocyte canalicular membrane, which is related to the multidrug resistance (mdr1b) gene. In several liver diseases the biliary transport is disturbed, resulting in jaundice and cholestasis for example. Many of these symptoms can be attributed to altered regulation of hepatic transporters (Roelofsen *et al*., 1997). The ultimate goal of the present study was to address the role of P-glycoprotein-mediated resistance and whether cyclosporin A could be used as a means to circumvent it.

In both hepatobiliary membrane and blood–brain barrier in which P-glycoprotein is reputedly expressed, the disposition of baicalein was affected by the P-glycoprotein modulator cyclosporin A as indicated by increased entry of baicalein into the brain and reduced excretion of levels of baicalein into the bile were observed. These results support previous observations demonstrating that disposition of baicalein is regulated by P-glycoprotein.

In conclusion, the tissues and blood pharmacokinetic data presented are important in confirming the expectation that baicalein is rapidly excreted into the bile and penetrate BBB 20–30 min after baicalein administration. In addition, the adding together of cyclosporin A shows significant effects on the pharmacokinetic parameters of baicalein with attenuation of the ratios of AUC_{bile}/AUC_{blood} and enhancement of the ratios of AUC_{brain}/AUC_{blood}. These results suggest that the hepatobiliary elimination and BBB penetration of baicalein might be regulated by P-glycoprotein.

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