Pharmacokinetic Effects of Baicalin on Cerebral Ischemia-reperfusion after iv Administration in Rats

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Abstract: **Objective** To investigate the pharmacokinetic effects of baicalin on cerebral ischemia-reperfusion (I/R) after iv administration in rats. **Methods** The cerebral I/R rats were induced by occluding the bilateral carotid arteries of normal rats for 2 h, followed by reperfusion. The resultant animals were immediately iv administrated with baicalin (90 mg/kg), whilst the same dose of baicalin was injected to the normal rats. Plasma samples were collected at different time to construct pharmacokinetic profiles by plotting drug concentration *vs* time. Quantification of baicalin in rat plasma was achieved using a simple and rapid HPLC method. **Results** In normal rats, the major parameters of distribution half-life, elimination half-life, area under the plasma concentration-time (AUC), apparent volume of distribution (V_d), and clearance (CL), estimated by an open two-compartmental model, were 0.8868 min, 26.0968 min, 149.6204 mg/min·L, 4.765 L/kg, and 0.5776 L/ kg·min), respectively. However, in I/R rats, the corresponding parameters were 2.084 min, 34.4998 min, 260.0188 μg·min/L, 5.9376 L/kg, and 0.334 L/(kg·min), respectively. **Conclusion** The cerebral I/R could significantly increase AUC and V_d values, decrease CL values, and prolong the terminal half-life of baicalin. These findings suggest that the injuries of I/R could play an important role in pharmacokinetic process of baicalin.

Key words: baicalin; cerebral ischemia-reperfusion; distribution half-life; elimination half-life; pharmacokinetics
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Introduction

Baicalin (chemical structure shown in Fig. 1), the main effective component of *Scutellariae Radix*, has been proved to increase the expression of Bcl-2 proteinum, reduce the expression of Bax protein in infectious brain group, relieve the degree of infectious cerebral oedema, and reduce the apoptotic cells of brain cortex in infectious brain oedema of rats (Liu, Wu, and Long, 2008). Moreover, baicalin possessed a protective effect on the cerebral ischemia-reperfusion (I/R) tissue by reducing nerve cell injury (Zhu *et al*, 2007) and decreasing the expressions of TNF-α and IL-1β (Yang *et al*, 2005), and reduced brain infarction volume and neurological deficit induced by transient ischemia insult (Xiong and Ouyang, 2007). Meanwhile, baicalin attenuated cerebral ischemia injury by down-regulating the expression of iNOS mRNA and COX-2 mRNA, and suppressed the elevation of caspase-3 protein and caspase-3 mRNA transcription in rats that underwent permanent middle cerebral artery occlusion (Liu *et al*, 2005; 2007; Tu, Yang, and Shi, 2009). Since baicalin has been demonstrated to exhibit so many pharmacological effects, the understanding of pharmacokinetics of baicalin is useful for designing an ideal dose regimen in pharmacological studies. Furthermore, the pharmacokinetic profile contributes to the safety and efficacy of baicalin in clinical application.

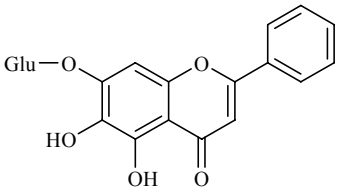

Fig. 1 Chemical structure of baicalin

The pharmacokinetic studies of baicalin have been previously published in several articles (Kotani *et al*, 2006; Lu *et al*, 2007). However, these investigations reported were performed in normal animals. So to

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investigate the pharmacokinetic effects of baicalin in pathology and their related mechanisms is needed (Zeng *et al*, 2010). It is well known that the pharmacokinetic process of a drug may be altered when the body is in pathosis, even significantly different from that in the normal conditions. Therefore, it is necessary to study the potential alterations of pharmacokinetic parameters under pathological conditions. Furthermore, the data obtained from pathological conditions could be more beneficial than those from normal conditions in clinical applications. The aim of this research is to study the difference of pharmacokinetic process in normal and pathological conditions and the pharmacokinetic effects of baicalin on cerebral I/R. To obtain the available pharmacokinetic data of baicalin, a simple and rapid HPLC method was developed for the determination of baicalin in plasma of rats.

Materials and methods

Chemicals and reagents

Baicalin (purity > 95%, 090212) was procured from Harbin Second Traditional Chinese Medicine Factory. The reference standard of baicalin (111022-20100812) and the internal standard (IS), rutin (111066-20100705, purity > 99%) were determined by HPLC analysis, which were provided by the National Institutes for Food and Drug Control (Beijing, China). Methanol (HPLC grade) was obtained from Sigma Co., Ltd. Phosphoric acid (AR grade) was bought from Guangzhou Chemical Reagent Co., Ltd. Double-distilled water was used for all preparations.

Animals

Adult male SD rats weighing 250－300 g were obtained from Experimental Animal Center of Guangzhou University of Chinese Medicine. Animals were kept in an environmentally controlled breeding room [temperature (25 ± 2) ℃, humidity (60 ± 5)%, 12 h dark/light cycle] for one week before the experiments. They were fed with standard laboratory chow with water *ad libitum* and fasted overnight. Experimental animals were maintained in accordance with internationally accepted principles for laboratory animal use.

HPLC conditions

The HPLC system consisted of a Waters 515 Pump, a Rheodyne 7725i Manual Injector, and a 996-Photodiode Array (PDA) UV-Vis Multi-wavelength Detector (Waters, USA). A Zorbax SB-C_{18} reversed-phase column (150 mm × 4.6 mm, 5 μm, Agilent, USA) was used. The signals from detector were collected and analyzed with a computer equipped with a Waters Millennium Chromatography Manager. The mobile phase was water-methanol-phosphoric acid (53:47:0.2), filtered through a 0.45 μm Millipore Filter and degassed prior to use. The flow-rate was 1.0 mL/min. The injection volume was 10 μL. Detection was performed at a wavelength of 280 nm under a constant temperature (30 ± 1) ℃.

Drug administration and preparation of plasma samples

Under anesthesia with 10% chloral hydrate (300 mg/kg), the cerebral I/R model rats were induced by isolating the bilateral common carotid arteries through a ventral midline incision in the neck, subsequently ligating and occluding the bilateral carotid arteries for 2 h, followed by restoring blood flow recirculation. The resultant rats were immediately administrated with baicalin (90 mg/kg) via femoral vein. Weight-matched normal rats were served as the normal control and injected with the same dose of baicalin. Blood samples (approximately 0.2 mL) were collected with heparinized syringes from each rat via abdominal aorta according to the specific schedule (0.5, 5, 10, 15, 30, 60, 90, 120, and 240 min after administration). Each collected blood sample was immediately transferred to a glass tube and centrifuged at 1500 × *g* for 15 min. The supernatant (0.1 mL) was then mixed with 1 mL methanol by vortexing for 1 min. The denatured protein precipitate was separated by centrifugation at 1500 × *g* for 15 min. The supernatant was evaporated at room temperature and then dissolved in methanol containing 20 μg/mL rutin. The volumes of methanol used were adopted to make the concentrations of baicalin in the samples which fell into the linearity scope of the standard curve. The sample solution (10 μL) was injected into HPLC for analysis. Data from these samples were used to construct pharmacokinetic profiles by plotting drug concentration *vs* time. The same sample handling process was used for the determination of recovery and precision of baicalin in plasma.

Calibration curve

A calibration curve was constructed based on the analysis of various concentrations of baicalin (0.1, 0.5, 1.0, 5.0, 10, 20, and 40 μg/mL) spiked in rat plasma

by HPLC. The concentrations of baicalin in the plasma were achieved by using the equation for linear regression obtained from the calibration curve.

Recovery

Plasma samples were spiked with three different concentrations (1, 10, and 100 μg/mL) of baicalin. The sample handling process was described as above. The resulting peak area ratios (baicalin / rutin) were compared with those of standards carried in methanol to provide the recovery values.

Precision

Precision in the working dose range was determined by triplicate analyses of plasma samples (n = 3) spiked with three different concentrations (1, 10, and 100 μg/mL) of baicalin. Intra-day variance was determined by assaying the same spiked samples at different times during the day. Coefficients of variation were calculated from these values.

Pharmacokinetic analysis

All data obtained were subsequently calculated based on the moment theory about the compartmental pharmacokinetic parameters of half-life ($t_{1/2}$), area under the plasma concentration-time curve (AUC), volume of distribution (V_d), and clearance (CL), processed by the computer program WinNolin 5.0.1 (Pharsight corporation, Mountain View, CA, USA).

Statistical analyses

All values were expressed as $\bar{X} \pm s$. Statistical analysis was performed using SPSS11.0 software. Student's t test was used to test whether the mean differed between two groups. Data were considered significant difference at $P < 0.05$.

Results

HPLC chromatograms

Under the condition described above, the typical HPLC chromatograms of blank plasma, plasma spiked with baicalin (10 μg/mL), and the plasma sample obtained 30 min after iv administration of baicalin are shown in Fig. 2. The retention times of baicalin and rutin were about 12.8 and 5.4 min, respectively. No interfering peaks were observed within the time frame in which baicalin and rutin were detected.

Calibration curves

The linearity of calibration curve was evaluated by analysis of peak area ratios (baicalin / rutin) to baicalin

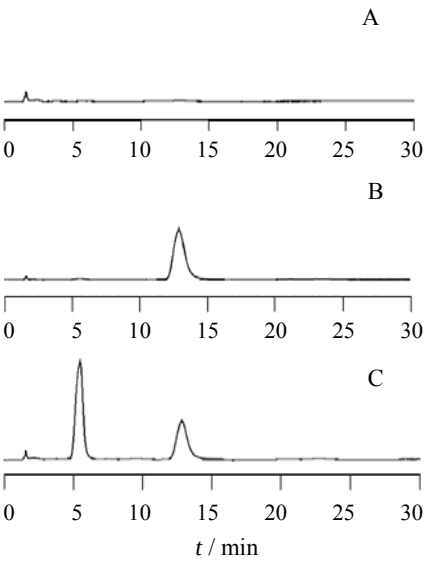

Fig. 2 Chromatograms of baicalin in blank plasma (A), blank plasma spiked with baicalin (10 μg·mL^{-1}) (B), and plasma sample obtained 10 min after iv administration of baicalin (C)

concentrations in rat plasma. The calibration curve for baicalin was linear (r^2 = 0.9992) over the concentration range of 0.1−40 μg/mL. A regression equation was as follows: $y = 26.135x + 0.3595$, where x represents peak area ratio of baicalin to rutin and y represents concentration of baicalin in plasma.

Recovery and precision

The recoveries of baicalin from rats plasma were 81.16%, 97.15%, and 91.19 % at concentrations of 1, 10, and 100 μg/mL, respectively (Table 1). The precision of the method was defined by examining intra-day variance. The RSD values of intra-day assay were 7.71, 5.86, and 2.58 at concentrations of 1, 10, and 100 μg/mL, respectively (Table 2). These validation results indicate that the method is suitable for the present study.

Determination of baicalin in plasma

The plasma concentration *vs* time profile of baicalin in rats is presented in Fig. 3. After iv administration of saline solution of baicalin, the plasma level of baicalin declined with a distribution half-life of 0.8868 and 2.084 min in normal and I/R rats, respectively. The concentration was lower than quantitative limit (0.1 μg/mL) after 4 h.

Kinetic analysis

As calculated from the plasma concentrations of baicalin following iv administration of baicalin, the pharmacokinetic parameters of baicalin are listed in Table 3.

Table 1 Recovery of baicalin ($\bar{X} \pm s$, $n = 3$)

Spiked concentrations	Peak area ratio		Recovery / %	RSD / %
	untreated	treated		
1	0.1815 ± 0.0064	0.1469 ± 0.005 548	81.16 ± 5.88	7.25
10	1.8628 ± 0.040 47	1.8080 ± 0.060 65	97.15 ± 4.95	5.09
100	17.547 97 ± 0.081 129	16.132 67 ± 0.036 506	91.94 ± 0.6	0.6

Table 2 Validation of intra-day assay ($\bar{X} \pm s$, $n = 3$)

Spiked concentrations	Measured concentrations	Recovery / %	RSD / %
1	0.80 ± 0.0616	80	7.71
10	10.6533 ± 0.6252	106.53	5.86
100	101.4933 ± 2.624 149	101.49	2.58

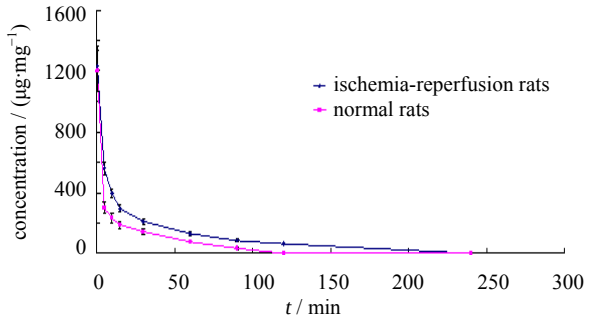

Fig. 3 Plasma concentration-time curve of baicalin in rats after iv administration (90 mg·kg^{-1} baicalin) ($\bar{X} \pm s$, $n = 5$)

Table 3 Pharmacokinetic parameters of baicalin (90 mg·kg^{-1}) in normal and cerebral I/R rat plasma ($\bar{X} \pm s$, $n = 5$)

Parameters	Unit	Estimate	
		Normal	I/R
K_{10}	min^{-1}	0.1214 ± 0.0058	0.0562 ± 0.0013**
K_{12}	min^{-1}	0.5588 ± 0.0902	0.2162 ± 0.0461**
K_{21}	min^{-1}	0.1446 ± 0.016	0.0988 ± 0.0029**
$t_{1/2\alpha}$	min	0.8868 ± 0.0929	2.084 ± 0.0494**
$t_{1/2\beta}$	min	26.0968 ± 1.5669	37.4998 ± 1.4543**
AUC_{0-t}	mg·min·L^{-1}	149.6204 ± 3.2414	260.118 ± 6.5028**
$AUC_{0-\infty}$	mg·min·L^{-1}	156.1302 ± 3.413	269.7578 ± 6.823**
V_d	L·kg^{-1}	4.765 ± 0.2176	5.9376 ± 0.0266**
CL	L·kg^{-1}·min^{-1}	0.5776 ± 0.0127	0.334 ± 0.0086**

$^{**}P < 0.01$ *vs* normal rats

Discussion

Rational drug therapy is dependent upon a basic understanding of the way that patients handle drugs (pharmacokinetics) and their response to specific drug effects (pharmacodynamics). Since knowledge of the pharmacokinetic processes could help us to explain and predict a variety of events related to the efficacy and toxicity of herbal preparations, it is important to do some pharmacokinetics investigations of baicalin for further evaluation of its clinical applications. Though there are many pharmacokinetic data about purified baicalin (Kotani *et al*, 2006) and baicalin in both *Scutellariae Radix* extract and compound prescription (Lu *et al*, 2007), there are no appropriate pharmacokinetic data of baicalin in pathological conditions, which may be more useful in providing the dose information and thus enhancing the safety and efficacy of baicalin in clinical applications. Moreover, since Chinese materia medica (CMM) is administered in abnormal condition in clinical practice, these related results obtained from normal conditions are not enough to reveal the clinical efficacy of baicalin.

In addition, recent studies showed that baicalin could be hydrolyzed and transformed to the easily absorbed baicalein, then be reabsorbed in gastrointestinal tract with the help of intestinal mucosa cell after administration (Gong and Yu, 2009). Therefore, it has a low bioavailability *in vivo*, which may be attributed to the first-pass metabolism in the gut wall or liver, metabolism or decomposition in the intestine by bacterial microflora, and/or poor absorption from gastrointestinal tract. To improve the clinical efficacy, it may be a better way to change the routine of administration such as from external venous to iv administration. In our work, the routine of iv administration was chosed, and the pharmacokinetic parameters of baicalin were evaluated after iv administration, implying that the great difference between normal and cerebral I/R model rats was observed. Though the concentration of baicalin declined rapidly and was no longer detected at 4 h post-injection in our study, we found that the plasma concentrations of baicalin in cerebral I/R rats were continuously higher than those in normal rats at sampling time points (0.5 5, 10, 15, 30, 60, 90, 120 min, $P < 0.01$). In brief, cerebral I/R could significantly prolong the half-life of distribution and elimination ($P <$

0.01), increase the AUC values ($P < 0.01$), and decrease the CL values ($P < 0.01$). The reasons resulting in the above alterations may be as follows: (1) The activity of some enzymes and the transfer ability of biomembrane were changed when the body was in pathological conditions; (2) The clearance rate of drugs decreased for the poor ability to remove drugs off at per time unit in the state of pathophysiology; (3) The mean retention time of baicalin in cerebral I/R rats was longer than that in normal rats, leading to the higher plasma concentrations of baicalin in cerebral I/R rats.

In conclusion, the metabolic rate of baicalin could decrease and the residence time could be prolonged *in vivo* in the state of cerebral I/R. Our findings suggest that the pharmacokinetic process of a drug will change in the conditions of disease, and the parameters obtained from the normal condition are limited and need to be modified according to the practice. In addition, the elimination rate of baicalin slowed down in cerebral I/R rats, which suggested that the drug will cumulate in the pathological state. Therefore, it is necessary to investigate the dynamics of drugs in patients to enhance the safety and efficacy of drug in clinical applications. The pharmacokinetic features of baicalin obtained from the present study could be applied as a reference for evaluating clinical efficacy of baicalin.

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