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Pharmacokinetics of baicalin-phospholipid complex in rat plasma and brain tissues after intranasal and intravenous administration

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The aim of this study is to determine whether baicalin can be transferred along the olfactory pathway to the brain after nasal administration of baicalin phospholipid (BP) complex to rats, thereby circumventing the blood brain barrier. The concentration of baicalin in plasma and different brain tissues (olfactory bulb, cerebral cortex, striatum and cerebellum) were measured by high-performance liquid chromatography (HPLC). The ratios of the area under the concentration-time curve (AUC) values of intranasal to intravenous administrations were 54.21 %, 240.59 %, 374.71 %, and 114.54 % in plasma, cerebral cortex, striatum, and cerebellum, respectively. In the olfactory bulb, the AUC values of intranasal to intravenous administrations were 3355.4 ± 378.8 μg/g·min versus 0 μg/g·min following intravenous administration. The ratios of AUC values of intranasal to intravenous administrations were72.75 %, 240.59 %, 374.71 %, 114.54 % in plasma, cortex, striatum, cerebellum respetively. The proportion of baicalin in the brain tissues from the olfactory transfer was also calculated, and the result shows that, following intranasal administration, approximately 52.36 %–100 % baicalin content at 8 h was transported to the brain via the olfactory pathway. In conclusion, the BP complex is transferred into the olfactory bulb via the olfactory pathway in rats, and the BP complex intranasal delivery is a promising approach to protect against cerebral ischemic injury.

1. Introduction

The blood brain barrier (BBB) represents a very complex endothelial interface that restricts the free diffusion of molecules and prevents the transport of most substances from the systemic circulation into the central nervous system to maintain a stable environment. However, the BBB is the primary obstacle to the delivery of therapeutic drugs to the brain, preventing many drugs from reaching it at therapeutic concentrations; lipid solubility, molecular mass, and charge of the drug molecules affect the extent to which they are absorbed from the blood into the brain. Therefore, brain-targeting delivery has been a great challenge in brain disease therapy in recent years (Illum et al. 2000, 2003a, b).

Intranasal administration is an attractive noninvasive route with advantages over oral or intravenous administration, such as higher bioavailability, rapid absorption, no first-pass hepatic metabolism, and ease of convenience. Recently, increasing studies have reported the possibility of circumventing the BBB to deliver drugs to the brain using the direct transport pathway from nose to brain via the olfactory region. Consequently, the nasal route has been considered as an important and promising way for drugs used in cerebral diseases. (Sakr et al.1996; Dhalin et al. 2001; Qizhi et al. 2004; Qiao et al. 2007; Ji-Ping et al. 2009).

Baicalin (7-D-glucuronic acid, 5,6-dihydroxy flavone), a glucuronide derived from the root of *Scutellaria baicalensis* Georgi, is widely used in traditional Chinese medicine for treating upper respiratory and gastrointestinal tract infections, viral hepatitis, and cardiovascular disorders (Duarte et al. 1993; Fukutake et al. 1998; Gao et al. 1999; Chan et al. 2000). In addition to displaying anti-oxidant activities, baicalin protects against cerebral ischemic injury by attenuating glutamate production (Lee et al. 2003; Li et al. 2003; Zhang et al. 2005).

However, oral or intravenous bioavailability of baicalin is very low because if its low lipid and water solubility, which causes low amounts of baicalin in the brain and limits its therapeutic effects and clinical application (Wang et al. 2005). To improve solubility and enhance the bioavailability of baicalin, a complex of baicalin and soy phospholipids was prepared at a weight ratio of 1:2 in a previous study (Bombardelli et al. 1992; Gatti and Peruua 1994; Bialecka et al. 1997; Elkheshen et al. 2001). *In vitro* permeation experiments on baicalin and baicalin phospholipid (BP) complex showed that the Papp value of BP complex was $(82.97 \pm 15.13) \times 10^{-7}$ cm·s^{-1}, approximately 2.32 times greater than that of baicalin ($P < 0.05$). In the past pharmacokinetic research, the AUC value of the group that received nasally administered BP complex was significantly higher than that of the group that received baicalin intravenously. Therefore, in this study, the pharmacokinetic behavior of baicalin is investigated in different brain tissues and in plasma following intranasal administration of the BP complex. The results are compared with intravenous administration to confirm whether there is a direct pathway for the BP complex from the nasal cavity along the olfactory mucosa into the brain.

2. Investigations and results

2.1. Validation of the HPLC method

As an internal standard, meletin shares weak acidic properties with the analyte baicalin. Under the conditions described above, both showed adequate separation with retention times of 8.5 and 12 min, respectively. No errant peaks on the chromatogram interfered with those of the analyte and the internal standard.
Calibration curves of baicalin were prepared with plasma and brain tissues mixed with known amounts of the drug, utilizing its HPLC peak area ratio to the internal standard. The linear range of baicalin was 0.0128–40 and 0.0384–120 μg/g, and inter- and intra-day variations were less than 6% and 10% for plasma and brain tissue samples, respectively. The extraction recoveries of baicalin from plasma and tissue homogenates were more than 85% and 75%, respectively.

2.2. Kinetic analysis

As calculated from the concentrations of baicalin in the samples following intranasal and intravenous administration of the BP complex, the main pharmacokinetic parameters are listed in the Table. The concentration-time profiles of baicalin in blood and brain tissues are presented in Fig. 1.

2.3. Drug targeting effiency (DTE)

Drug targeting effiency (DTE) in different brain tissues was calculated according to Eq. 1. The results are shown in Fig. 2.

3. Discussion

Similar to most components obtained from traditional Chinese herbs, baicalin possesses low lipid and water solubility, which causes low permeation into biological membranes. In a previous study, these conditions have been improved by preparing baicalin into phospholipids complex. Considering BP complex has exhibited higher permeation of the olfactory mucosa and brain-concentration than baicalin, the pharmacokinetics of baicalin-phospholipid complex in rat plasma and brain tissues after intranasal and intravenous administration have been investiged in this study, therefore, to study the existence of nose-brain direct transport pathway for phospholipid complex.
Following intranasal administration, the highest concentration was observed in the olfactory bulb (the peak drug level was 38.34 ± 6.06 μg/g), followed by the cortex (the peak drug level was 2.821 ± 0.952 μg/g), the striatum (the peak drug level was 32.658 ± 0.288 μg/g), and cerebellum (the peak drug level was 1.555 ± 0.285 μg/g). As shown in the Table, baicalin was particularly concentrated in the olfactory bulb after nasal administration, the $AUC_{0\rightarrow480}$ value was 3355.4 ± 378.845 versus 0 μg/·min obtained after intravenous injection; this value was also considerably higher in the cortex, striatum, and cerebellum compared with intravenous administration. In this study, the drug could not be detected in the olfactory bulb after intravenous injection. This might be due to the low mass of the sample. After intravenous administration, the drug permeated the BBB and was then distributed to different brain tissues. The olfactory bulb was the smallest sample, with an average mass of approximately 0.07 g; thus, its baicalin content was very low.
The ratios of AUC values of intranasal to intravenous administrations were 72.75%, 240.59%, 374.71%, 114.54% in plasma, cortex, striatum, cerebellum, respetively.
It was assumed that if drug concentration in the brain was significantly higher after intranasal administration than after intravenous administration, a direct pathway from the nasal olfactory area into the brain must exist for this drug.

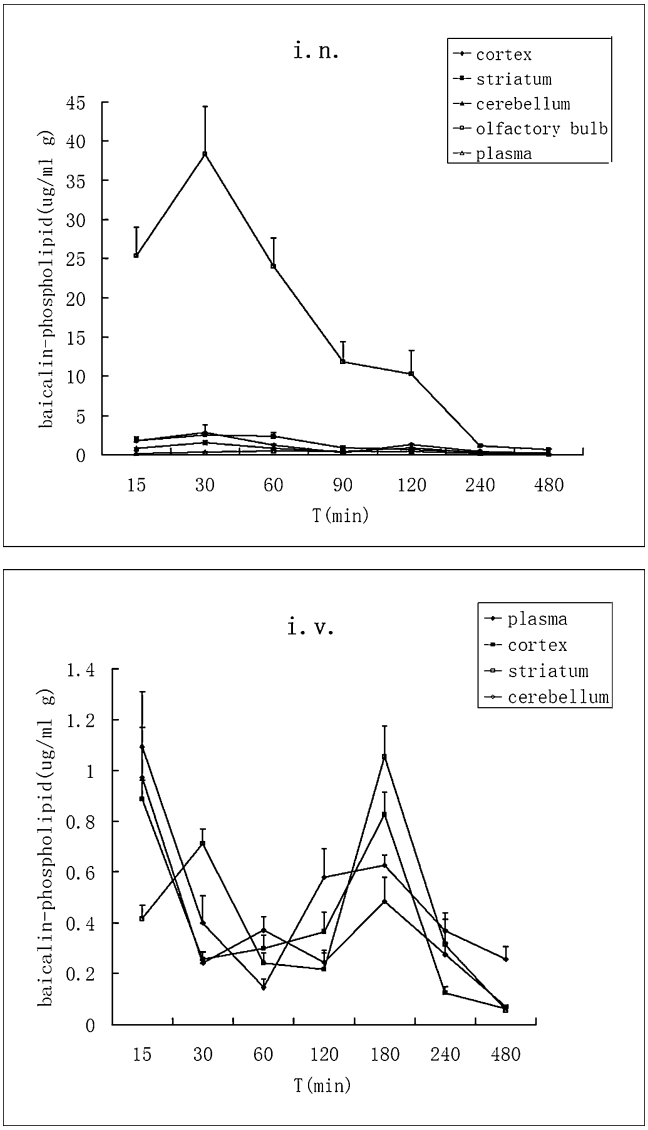

Fig. 1: Plasma and brain tissue concentration-time profiles of baicalin after nasal administration or intravenous administration of baicalin-phospholipid complex in rats (n = 5)

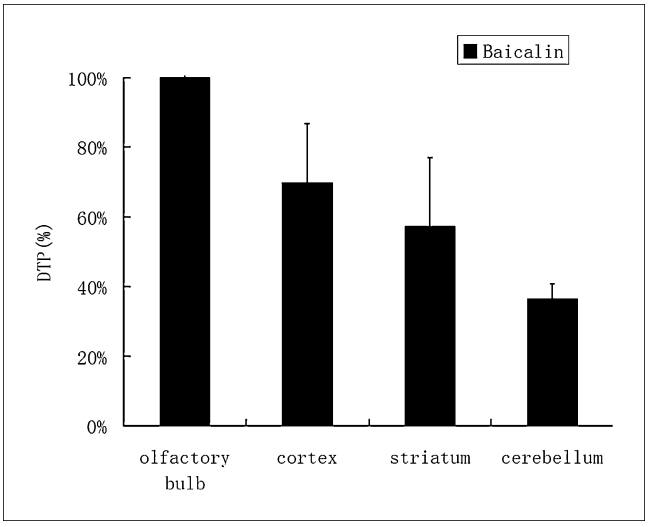

Fig. 2: Olfactory proportion after nasal administration of baicalin-phospholipid complex. Data represent the mean ± S.D

Table: Pharmacokinetic parameters following intravenous and intranasal administration of baicalin-phospholipid complex

Parameters	Route	Plasma	Olfactory bulb	Cortex	Striatum	Cerebellum
C_{max} (μg/ml g)	i.n.	0.831 ± 0.072	38.34 ± 6.06	2.821 ± 0.952	2.658 ± 0.288	1.555 ± 0.285
	i.v.	1.093 ± 0.217	0	0.886 ± 0.074	1.053 ± 0.122	0.972 ± 0.199
T_{max}(min)	i.n.	120	30	30	42 ± 16.432	30
	i.v.	15		15	120	15
$AUC_{0\sim480}$ (μg/g*min)	i.n.	148.705 ± 10.863	3355.4 ± 378.845	329.234 ± 17.001	313.698 ± 19.708	158.331 ± 4.325
	i.v.	204.406 ± 17.509	0	136.843 ± 12.954	183.717 ± 22.389	138.229 ± 10.68
Ratio of $AUC_{i.n.}/AUC_{i.v.}$(%)		72.750		240.59	374.71	114.54

Statistical difference was calculated using Student's t-test. $P < 0.05$. Data represent the mean ± S.D. i.n. (n = 5); i.v. (n = 5)

Following intravenous administration, plasma baicalin concentrations peaked at 15 min compared with intranasal administration, which achieved its maximum concentration after 120 min. Considering that, after intranasal administration, one part of the drug absorption into the systemic circulation takes place across the nasal respiratory mucosa, this administration method prolongs the T_{max} of the drug.

After intravenous injection, the T_{peak} in the cortex and cerebellum was 15 min; in striatum, the maximum concentration was observed at 120 min. Following intranasal administration, the profiles of the baicalin level in the brain displayed an initial absorption phase, and maximum concentration was achieved after approximately 30 min in the olfactory bulb, cortex, and cerebellum, and 42 min in the striatum.

The whole nasal cavity is covered with respiratory and olfactory mucosa; the former is richly vascularized and highly perfused. Following nasal administration, some of the drug reached the respiratory mucosa and is absorbed into the systemic circulation and subsequently reaches the brain by crossing the BBB; in contrast, some of the drug can move from the olfactory region directly into brain tissue. The olfactory proportion represents the percentage of the drug directly transported into the brain via the olfactory pathway. In this study, the olfactory proportion was calculated to illustrate the nose-brain direct transport more clearly following nasal administration of BP complex. The results show that following intranasal administration, approximately 52.36–100% of the baicalin content at 8 h was transported to the brain via the olfactory pathway.

The results indicate that after intravenous administration of BP complex, the drug can move from the nasal mucosa directly into brain tissue. As a traditional Chinese medicine for treating cerebral disease, intranasal administration can potentially replace the common routes of administration.

4. Experimental

4.1. Materials

Baicalin reference substance was obtained from the National Institute for the Control of Biological and Pharmaceutical Drugs (P.R. China), Batch No.:110715-200514; Meletin was obtained from the National Institute for the Control of Biological and Pharmaceutical Drugs (P.R. China), baicalin extract (97.2%, MW = 446.35) was purchased from Shanghai Bo'ao Biological Technology Co., Ltd. (P.R. China); Soybean Lecithin (nitrogen content > 99.8%) was purchased from Kelong Chemical reagent plant in Chengdu (P.R. China); HPLC-grade methanol, analytical reagent-grade phosphoric acid, and potassium dihydrogen phosphate were used for analysis. Double-distilled water was produced in this laboratory.

4.2. Preparation of baicalin-phospholipid complex

The complex was prepared with baicalin and soy phospholipids at a weight ratio of 1:2. Weighed amounts of baicalin and soy phospholipids were placed in a 1000 ml round bottom flask and 500 ml of tetrahydrofuran was added to produce baicalin concentration to 2.5 mg ml^{-1}. The mixture was refluxed (mixed with magnetic force) in a thermostatic water bath at a temperature of 55 °C for 1 h. The resulting clear solution was evaporated to remove traces of solvents at 50 °C *in vacuo*. The BP complex were obtained in the form of a yellowish powder. The complex was standardized to 40 % baicalin using HPLC method.

4.3. Nasal absorption and brain distribution studies

4.3.1. Animal experiment

Female Sprague-Dawley rats (200–250 g) were obtained from the Experimental Animal Center of Chengdu University of Traditional Chinese Medicine and kept in an environmentally controlled breeding room with an ambient temperature of 20 ± 2 °C and 60% ± 5% humidity for 1 week. They were randomly assigned into seven groups according to time points. Measurements were made using five rats at each time point. After a 12 h fast, mice were given access to water prior to the nasal administration of the BP complex. They were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg), and kept on a heating pad to maintain the body temperature. The trachea was cannulated with a polyethylene tube (PE 200) to allow free breathing. An incision was made in the skin over the occipital bone. The first layer of muscle was cut, and the atlanto-occipital membrane was exposed. All of the incisions were covered with wet gauze. For the intranasal administration, 200 μl of the 60 mg/ml BP complex aqueous solution was administered via a PE 200 tube attached to a microliter syringe inserted 1 cm into each nostril of rat approximately 30 min after operation. For the intravenous administration, the BP solution was delivered (dose equivalent to 12 mg) through the femoral vein and the injection volumes were 0.8 ml. After dosing for 0.25, 0.5, 1, 1.5, 2, 4, 6, and 8 h, blood was collected from the femoral artery with a polyethylene pipe. The blood samples were then centrifuged at 12000 rpm for 10 min. The plasma (0.6 ml) was transferred to a 5 ml centrifuge tube and mixed with 0.3 ml of 1 mol/l potassium dihydrogen phosphate, 0.9 ml extraction solvent consisting of methanol-acetonitrile (50:50, v/v%), and 0.1 ml internal standard solution of meletin at a concentration of 30 μg/ml. The mixture was vortexed for 4 min and centrifuged at 12000 for 4 min. The supernate was transferred to another PE conical tube as plasma sample. The animals were decapitated immediately after blood was collected. Then, the skull was cut open and the olfactory bulb, cortex, striatum, and cerebellum were carefully excised. Each sample of brain tissue was quickly rinsed with saline and blotted with filter paper remove as much of the blood and macroscopic blood vessels as possible. After weighing, the brain tissue samples were homogenized with 1 mol/l potassium dihydrogen phosphate with an extraction solvent consisting of methanol-acetonitrile (50:50, v/v%). Brain tissues homogenates (0.5 ml) were transferred to a 5 ml centrifuge tube with 0.1 ml meletin added. The mixture was vortexed for 4 min. After centrifugation at 12000 rpm for 10 min, the supernate was transferred to a conical tube as brain tissue samples. All samples were evaporated to dryness under a stream of nitrogen at 35 °C and stored for up to 24 h in a freezer (–70 °C) until HPLC analysis. Measurements were made using five rats at each time point. The experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Westin et al. 2005; Zhenqi Shi et al. 2005).

4.3.2. Analytical procedures

Baicalin in plasma and brain tissue was assayed according to a modified HPLC method. The residue was dissolved in 200 μl of methanol, centrifuged at 12000 rpm for 10 min, and 20 μl of the solution was injected into an HPLC system consisting of a Shimadzu LC-10AT pump, a Shimadzu LC-10AT autosampler, and a Shimadzu SPD-10A VWD detector (Shimadzu, Japan). Chromatographic separation was achieved at ambient temperature on a 4.6 × 250 mm C18 analytical column (SHIMADZU VP-OD). For plasma and brain tissue samples, the mobile phase was 47% double-distilled water, 53% methanol, 0.2% phosphoric acid (v/v) at a flow rate of 1 ml/min and a detection wavelength of 280 nm (Lujun Zhang et al. 2006)

4.3.3. Data analysis

Results obtained from the HPLC analyses are plotted as drug concentration-time curves in plasma, olfactory bulb, cortex, striatum, and cerebellum. AUC values were calculated from the time zero to the last data point using the trapezoidal method without extrapolation to infinity. Student's t-test was used to study the statistical differences and a value of $P < 0.05$ was considered statistically significant. Results are presented as mean values±S.D.
The proportion of baicalin in the brain tissues due to olfactory transfer was calculated according to Eq. 1 (Qizhi Zhang et al. 2004).

$$Olfactory\ proportion = \frac{(AUC_{observed} - AUC_{\exp\ ected})}{AUC_{obserbed}} \bullet 100 \quad (1)$$

The $AUC_{expected}$ was defined as the AUC $_{expected}$ if there was no direct olfactory contribution to the baicalin concentrations in the brain. This was calculated as the fraction of the dose entering the brain after intravenous administration (the brain: plasma AUC ratio) multiplied by the nasal plasma AUC. The observed AUC was the AUC after nasal administration.

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