

Intranasal administration of baicalin-phospholipid complex improves brain targeting and ischemic-reperfusion injuries

Nan Li¹, Jia-Hua Ma², Ming Yang^{3*}, Ying-Jie Ye¹, Run-Chun Xu¹

1 Chengdu University of T.C.M, No.1166, Liu-tai Road, Wenjiang District, Chengdu 611137, PR China

2 School of life science and engineering, Southwest University of Science and Technology, No.59, Qinglong Road, Mianyang, 621010, P.R.China

3 Key Laboratory of Modern Preparation of T.C.M, Ministry of Education, No.18, Yun-wan Road, Wanli District, Nanchang 330004, PR China

Abstract

The aim of this study was to increase the concentration of baicalin in the brain tissue, and improve its therapeutic effect on brain diseases. Firstly, the microscopic observation and infrared spectroscopy (IR) were employed to confirm the formation of baicalin-phospholipid complex (BP complex). Then, the solubility, in vivo nasal absorption and olfactory transfer of BP complex after nasal administration which compared with baicalin and physical mixture were also investigated. The results proved that BP complex possessed better solubility and nasal mucosa absorption than baicalin or physical mixture, and also we proved that after nasal administration, a direct pathway for the transport of the three samples (baicalin, physical mixture, BP complex) to the brain via the olfactory mucosa existed. Finally, the results of the pharmacological evaluation indicated intranasal administration of BP complex could significantly reduce neural injury and brain edema.

Keywords: baicalin, phospholipid complex, intranasal administration, solubility study, nasal mucosa absorption, olfactory transfer study, pharmacological evaluation

1 Introduction

Baicalin, which belongs to glucuronides, is an effective constituent derived from the Chinese medicinal herb *Radix Scutellariae*. In China, it has been widely used for treating various diseases such as upper respiratory, gastrointestinal tract infection and cardiovascular disorders¹⁻³. Recent studies have demonstrated that baicalin has a protective effect against brain edema and cerebral ischemic damage^{4,5}.

However, after oral or intravenous administration, the concentration of baicalin in the brain tissue is very low due to its low lipid and the difficulty of penetrating the blood brain barrier (BBB). In this study, a phospholipid complex was adopted to increase both the water solubility and liposolubility of baicalin to enhance its ability of membrane-transportation⁶. In previous study, we prepared the BP complex, and in this paper, the microscopic observation and infrared spectroscopy (IR) were employed to confirm the formation of BP complex. And the solubility of BP complex which compared with baicalin and physical mixture were investigated.

Considering the previous studies reported that the intranasal administration possessed the advantages in the cerebral diseases therapy (nose-brain direct route, being

absorbed rapidly in respiratory region of the nasal mucosa)⁷⁻¹⁰; in this study, the pharmacokinetic behavior of baicalin in brain tissue and plasma following intranasal administration of three samples (BP complex, baicalin and physical mixture) has been investigated, the results are compared with intravenous administration. Furthermore, we also employed the model of focal cerebral ischemia in rats to investigate the effects of the three samples on brain edema through intranasal or intravenous administration.

2 Materials and methods

2.1. Materials and animals

2.1.1 Animals

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 wk.

2.1.2 Phytochemicals

Baicalin and meletin reference substance was purchased from the National Institute for the Control of Biological and Pharmaceutical Drugs (PR China), Batch No.:110715-200514; Baicalin Extract (97.2%, MW= 446.35) was purchased from Shanghai Bo'ao Biological Technology Co., Ltd. (PR China); Soybean Lecithin (nitrogen content >99.8%) was purchased from Kelong Chemical reagent plant in Chengdu (PR 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.

2.1.3 Chromatographic conditions

Chromatographic separation was achieved at ambient temperature on a 4.6 × 250 mm C18 analytical column (SHIMADZU VP-OD). 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. The perfusion samples were used UV spectrophotometer at wavelength of 280 nm.

2.2. 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 85% baicalin using HPLC method.

2.3 Preparation of physical mixture

The physical mixture was prepared with baicalin and soy phospholipids at a weight ratio of 1:2, and then blend them together, the physical mixture was obtained.

2.4 Characterization of BP complex

2.4.1 Microscopic view of the complex

The BP complex was suspended in distilled water and a drop was placed on a slide and covered with a cover slip. Microscopic view of the sample were observed at a magnification of 1500× and 10000×

2.4.2 Fourier Transform Infrared Spectrophotometry (FT-IR)

The FT-IR spectrometer (BRUKER IFS-55, Switzerland) was used to study the interaction between baicalin and phospholipids. The IR spectra of baicalin, phospholipids, the BP complex, and the physical mixture were obtained by the KBr method. The frequency range was 4,000–450 cm⁻¹.

2.5 Solubility studies

Solubility determination of baicalin ,BP complex and the physical mixture were carried out by adding excess of them into 10 ml of water or *n*-octanol in sealed glass containers at 25°C separately. The liquids were agitated for 24 h, then centrifuged at 4000 rpm for 15 min. The supernatant was filtrated through a 0.45 μm membrane. The 1 ml (filtrate was added in 10 ml flask, then add methanol to the mark.) and a 20 μl aliquot of the resulting solution was injected into a HPLC and detected at a wavelength of 280 nm, the concentration of baicalin was measured.

2.6 In vivo nasal absorption study

2.6.1 Animal experiment

Sprague–Dawley female rats are randomly assigned into three groups , baicalin group, BP complex group and physical mixture (baicalin:phospholipid=1:2 w/w) group. The rats are anesthetized by i.p. injection of 50 mg/kg sodium pentobarbital. After an incision was made in the neck of the rats, the trachea was cannulated with a polyethylene tube. Another tube was inserted through the esophagus to the posterior part of the nasal cavity. This tube served to introduce the perfusing solution into the nasal cavity. The nasopalatine was closed with an adhesive agent to prevent the drainage of the drug solution from the nasal cavity into the mouth. 5 ml drug solutions with concentration equivalent to 0.4 mg/ml baicalin were placed in a water jacketed beaker and kept at (37±0.2)°C by means of a circulating water bath. The perfusion solution passed out from the nostrils through the funnel and into the beaker again . polystaltic pump was at a rate of 2 ml /min. Serial samples (0.6 ml) , taken at following times: 0, 10, 20, 30, 40, 50, 60, 90 min, were added to in 25 ml flask, then add buffer to the mark. The results obtained from the HPLC were used to calculate the absorption of baicalin

2.6.2 Analytical procedures

Baicalin was assayed with the 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

2.6.3 Data analysis

The value of *k* reflects the extent of the mucous membrane absorption of the drug

$$\ln C = \ln C_0 - kt \quad \text{Eq.1}$$

where *C*₀ represents the initial concentration of the drug. *C* is the concentration of the rest drug . *k* represents absorption rate.

2.7 Olfactory transfer study

2.7.1 Animal experiment

Animals were randomly assigned into six groups according to time points. Measurements were made using five rats at each time point. After a 12 h fast, rats were given access to water prior to the nasal administration. 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.

Donor solutions were prepared by dissolving the baicalin, BP complex and physical mixture into water, respectively. The baicalin concentration of donor solutions for intranasal administration were 20 mg/ml, the 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 baicalin concentration of the donor solutions were 4 mg/ml, and the injection volumes were 1 ml. After dosing for 2, 5, 15, 120 and 240 min, 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 brain 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 (1.5 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.

2.7.2 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

2.7.3 Data analysis

Results obtained from the HPLC analyses are plotted as drug concentration-time curves in plasma, brain. 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 \pm S.D. The proportion of baicalin in the brain tissues due to olfactory transfer was calculated according to Equation 1

$$\text{Olfactory proportion} = \frac{(\text{AUC}_{\text{observed}} - \text{AUC}_{\text{expected}})}{\text{AUC}_{\text{observed}}} \cdot 100 \quad \text{Eq.2}$$

The $\text{AUC}_{\text{expected}}$ 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.

2.8 Pharmacological evaluation of BP complex against focal cerebral ischemia-reperfusion (I/R) injury

2.8.1 Groups and administration

Eighty male Sprague-Dawley rats were used in this experiment. They were randomly divided into 8 groups (10 rats per group). Group 1, the sham operation group, was given physiological saline. Group 2, the cerebral ischemia model group, was also given physiological saline. Group 3 was injected with baicalin solution through intravenous administration. Group 4 was intranasally administered baicalin solution. Group 5 was injected with BP complex solution through the intravenous way. Group 6 was intranasally administered BP complex solution. Group 7 was injected with the physical mixture through the i.v.administration. Group 8 was intranasally administered the physical mixture. Drugs were given three times in each group; each rat in the nasal administration group received 0.2 ml. Rats receiving drugs through the i.v.administration were injected with 1 ml. For the intranasal administration, the concentration of baicalin in baicalin solution, physical mixture solution or BP complex solution were 20mg/ml. For the intravenous administration, the concentration of baicalin in baicalin solution, physical mixture solution or BP complex solution were 4mg/ml. Each group was treated with drugs before the operation, 2 h after the operation, and 23 h after the operation.

2.8.2 Establishment of cerebral I/R model rats

Rats were anesthetized with 10% chloral hydrate (350 mg/kg i.p.) Brain I/R injury was induced by middle cerebral artery occlusion (MCAO) using the nylon suture method described previously. The right common carotid artery, external carotid artery (ECA), and internal carotid artery (ICA) were isolated via a ventral midline incision. A 50 mm length of monofilament nylon suture (Φ 0.22–0.24 mm), with its tip rounded by heating near a flame, was introduced into the ECA lumen and advanced into the ICA (about 18–22 mm deep) to block the origin of the MCA. The body temperature of the rats was maintained at 37 ± 0.5 °C during the surgical procedure with an infrared heat lamp. Sham-operated animals were not exposed to I/R. After 2 h of ischemia, the nylon suture was withdrawn to establish reperfusion. After arousal from anesthesia, the rats were returned to cages.

2.8.3 Neurological scores¹¹⁻¹⁴

After 22 h of reperfusion, motor responses were scored on a five-point scale according to Longa: 0 indicated no neurologic deficit, 1 indicated failure to extend left forepaw fully, a sign of a mild focal neurologic deficit, 2 indicated circling to the left, a sign of a moderate focal neurologic deficit, and 3 indicated falling to the left, a sign of a severe focal deficit. Rats with a score of 4 did not walk spontaneously and had a depressed level of consciousness.

2.8.4 Determination of water content after cerebral I/R

To evaluate brain edema, the rats were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg). After 24 h of reperfusion, the brains were rapidly removed and the wet weight of the brain was measured. The contralateral and ischemic hemispheres were then dried at 105 °C for 24 h, and the dry weight of each hemisphere was measured. The water content in the brain was calculated from the weight difference between the wet and dried tissue.

2.8.5 Statistical method

The results were expressed as mean \pm SD. Data were expressed and analyzed with SPSS13.0. Statistical analysis was performed by Student's t-test and ANOVA. Differences were considered significant for values of $p < 0.05$.

3 .Results and discussion.

3.1. Microscopic observations

The microscopic view, as shown in Fig.1 and Fig.2, indicated the presence of spherical structures of the complex. The vesicles consisted of soy phospholipids, and baicalin was intercalated in the lipid layer.

3.2 FT-IR

The infrared spectra of baicalin, phospholipids, their physical mixture, and the BP complex are illustrated in Figure 3. A significant difference between the spectrum of baicalin alone (Fig. 3A) and phospholipids alone (Fig. 3B) was obviously seen. The spectrum of the physical mixture (Fig. 3D) was similar to that of baicalin, but the spectrum peaks of phospholipid at $1,740\text{ cm}^{-1}$ and $1,460\text{ cm}^{-1}$ were still present. The spectrum of the physical mixture showed an additive effect of baicalin and phospholipids. There was significant difference between the spectra of the physical mixture and the complex (Fig.3C), and the baicalin spectrum also exhibited differences with the complex spectrum. The characteristic absorption peaks of baicalin at $3,300\text{--}3,600\text{ cm}^{-1}$ disappeared in the complex, while the characteristic absorption peak of $\nu\text{C=O}$ at $1,600.96\text{ cm}^{-1}$ shifted to $1,667.62\text{ cm}^{-1}$. The benzene skeleton vibration peaks of baicalin ($\nu_{\text{C=C}}$, $1,609.13$, $1,573.41$, $1,551.97$,

Fig 1 Microscopic views of baicalin–phospholipid complex with a magnification of $1500\times$.

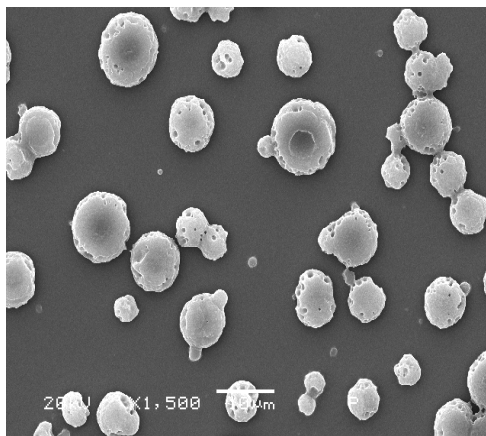
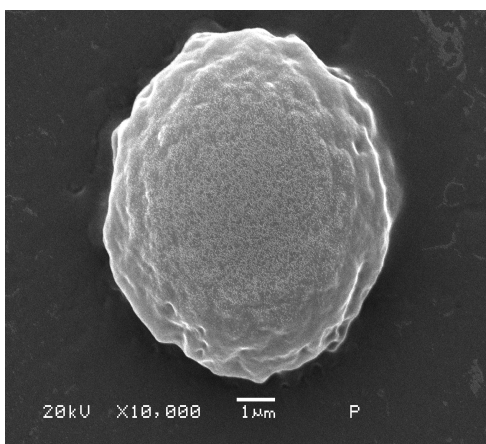


Fig 2 Microscopic views of baicalin–phospholipid complex with a magnification of 10000 \times .



1,496.32 cm^{-1}) also significantly changed, with the peak at 1,609.13 shifting to 1,617.79 cm^{-1} . The peaks at 1,573.41 and 1,551.97 cm^{-1} disappeared, replaced by a new peak at 1,587.58 cm^{-1} . The spectrum peaks of phospholipids of $\nu\text{C-H}$ at 2,925.61 and 2,854.13 cm^{-1} (for $\nu\text{C=O}$ stretching vibration), and δCH_2 at 1,466.36 cm^{-1} (for δCH_2 bending vibration) showed no change. These results suggested that BP complex was formed by weak physical interactions between baicalin and phospholipids. Thus, the physical mixture should show weak interaction, such as the combination of hydrogen bonds or van der Waals force.

3.3 Solubility studies

Tables 1 shows the solubility of baicalin, physical mixture and BP complex in water and n -octanol. The result showed that the BP complex has increased the solubility of baicalin in water and in octanol, due to the strong dispersibility or/and amorphous form of the phospholipids complex.

3.4 In vivo nasal absorption study

As Fig.4 shows, nasal absorption of all three samples in rats changed little after 40 ~ 50min, close to the absorption saturation. However, the cumulative absorption of baicalin- phospholipid complex was significantly higher than that of baicalin and physical mixture before 30 min, especially in 10 min. Both values of nasal mucosa

absorption by the t test showed significant difference ($p < 0.05$) The results demonstrated the form of complexes can significantly affect the absorption rate to Fig 3The infrared spectra of baicalin(A), phospholipids(B), complex(C) and physical mixture(D)

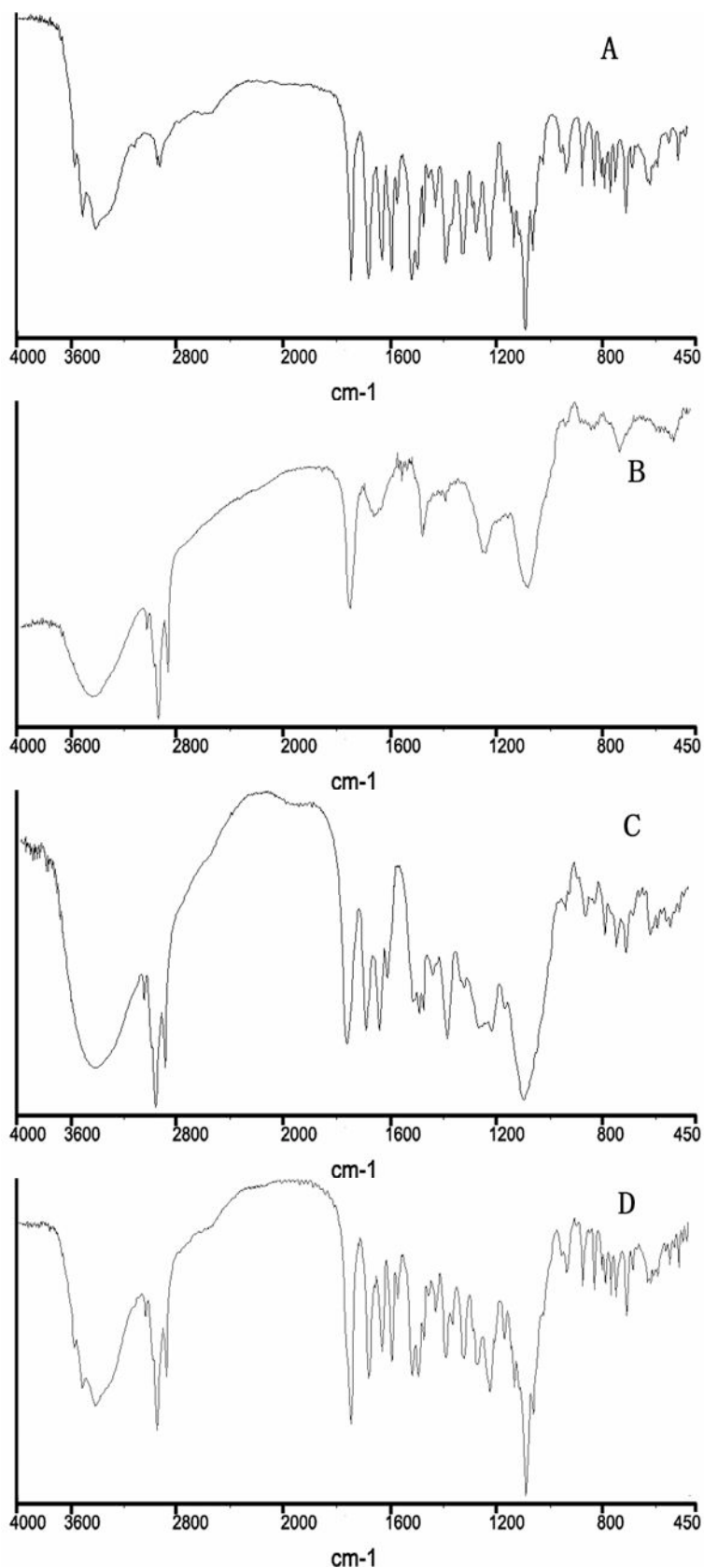


Fig 4 In vivo nasal absorption of baicalin, physical mixture and BP complex

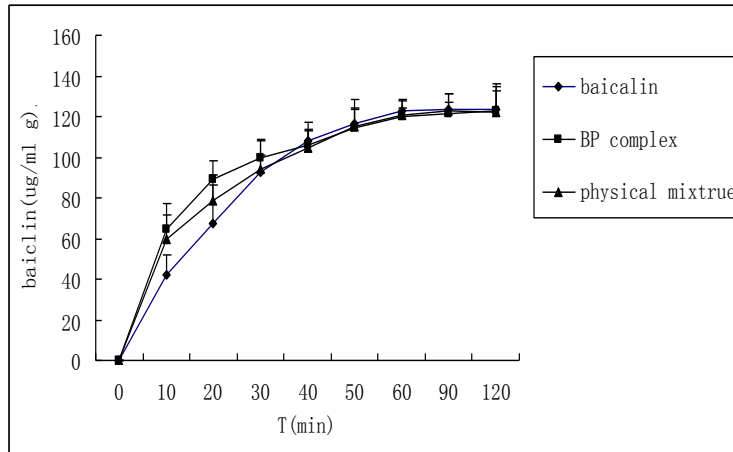
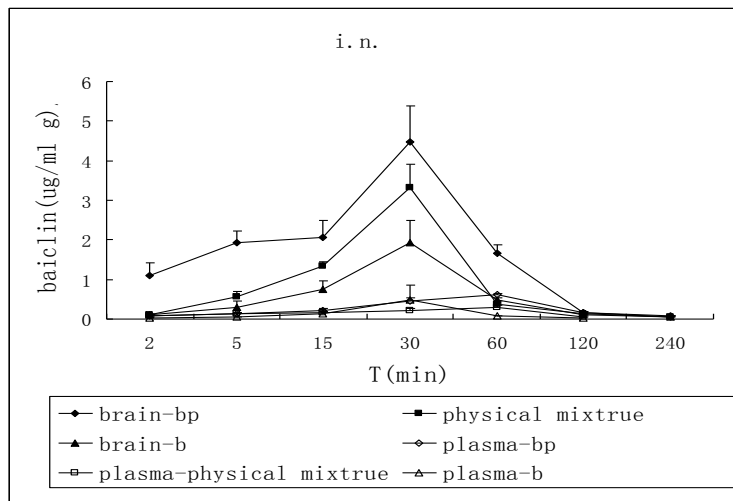
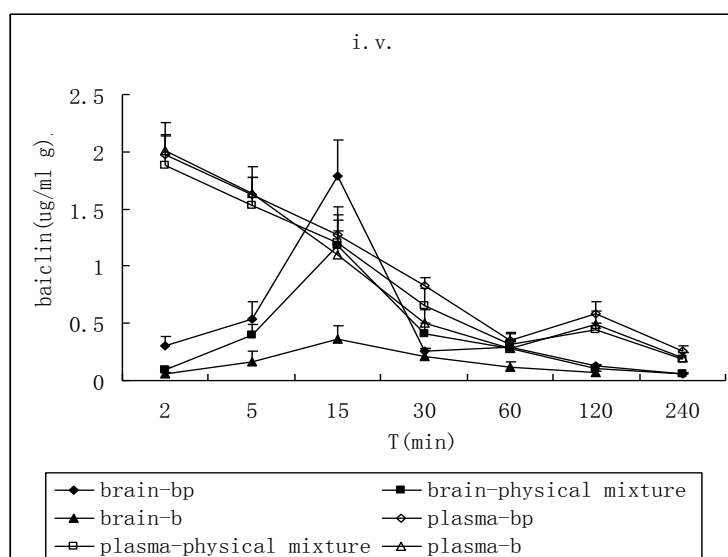


Fig.5 Pharmacokinetic parameters following intravenous and intranasal administration of baicalin, physical mixture and BP complex





promote the absorption of baicalin, before reaching the absorption equilibrium. As the cumulative absorption of baicalin from 10min to 50min showed obvious increasing trend, this segment demand the linear regression to get nasal mucosa absorption rate k : baicalin-phospholipid complex is $5.37 \pm 2.04 \times 10^{-3}/\text{min}$, physical mixture is 3.44 ± 1.52 and baicalin is $3.04 \pm 1.34 \times 10^{-3}/\text{min}$. The results showed that, in 10min ~ 50min range, the k of baicalin-phospholipid complex is significantly greater than that of baicalin and physical mixture, which clearly demonstrated the form of complex could significantly increased the in vivo absorption and also the phospholipids can promote the absorption of baicalin.

3.5 Olfactory transfer study

The whole nasal cavity is covered with olfactory and respiratory mucosa. For the olfactory mucosa, drugs could travel from this region directly into brain tissue. The result of this study indicated that following intranasal administration, the concentration of baicalin in the brain were significantly higher after intranasal administration than during intravenous administration; thus, we assumed that a direct pathway from the nasal olfactory area of the brain must exist for baicalin. In this study, the olfactory proportion of the each group was calculated to illustrate the nose-brain direct transport more clearly. The results show that following intranasal administration, the olfactory proportion for baicalin, physical mixture and BP complex were 79.85%, 58.11%, 74.17% separately.

After intranasal administration, the level of AUC in brain of the complex group was significantly higher than the baicalin group or the physical mixture group, the result indicated the form of BP complex could enhance the absorption on the olfactory mucosa of baicalin. Besides the olfactory mucosa, the nasal cavity is covered with respiratory mucosa, it is richly vascularized. On the respiratory region, drugs could be absorbed into the systemic circulation. In this study, the result shows after formulation of BP complex, the bioavailability of baicalin following intranasal administration was significantly increased compared with baicalin and physical mixture group. When

following intravenous administration, the drug must permeate the BBB, then get into the brain tissue. The result showed that after the intravenous administration, the level of AUC in brain of the complex group was remarkably increased comparing with the baicalin group or the physical mixture group. And we also discovered that the membrane-transportation of BP complex was significantly higher than the physical mixture. Therefore, we concluded that the form of complex can dramatically improve baicalin's transportation ability acrossing the biologic membrane (the nasal mucosa and the BBB), the reason might be that the BP complex could dramatically improve the membrane-absorption of baicalin, and not just through the action of phospholipids; the change of the crystal form, good solubility and permeability may be the main reasons for the improved absorption.

In addition, the previous research indicated that following the oral administration, the bioavailability was 4~6%, in this study, after the intranasal administration, the bioavailability has been increased to 15.5%. In the previous study reported that baicalin could treat various diseases such as upper respiratory and gastrointestinal tract infection and viral hepatitis. Therefore, the result of this study suggests that intranasal administration is a good choice to treat the diseases which mentioned above.

Table1 Solubility of baicalin, physical mixture and BP complex in water and *n*-octanol at 25 °C

Samples	Solubility in water(ug/ml)	Solubility in <i>n</i> -octanol(ug/ml)
Baicalin	126.93±1.74	68.96±2.21
BP complex	578.88±1.88	4839.525±2.08
Physical mixture	182.45±1.44	88.69±1.33

Values are mean± SD (n=5)

Table2 In vivo nasal absorption of baicalin, physical mixture and BP complex

Samples	k(min ⁻¹)
Baicalin	3.04±1.34·10 ⁻³
BP complex	5.37±2.04·10 ⁻³
Physical mixture	3.44±1.52·10 ⁻³

Values are mean± SD (n=5)

Table 3. Pharmacokinetic parameters following intravenous and intranasal administration of baicalin, physical mixture and BP complex

Group	Route	Plasma			Brain		
		C _{max} (μg /ml g)	T _{max} (min)	AUC ₀₋₂₄₀ (μg /g*min)	C _{max} (μg /ml g)	T _{max} (min)	AUC ₀₋₂₄₀ (μg /g*min)
baicalin	i.v.	2.01±0.136	2	110.597±12.275	0.362±0.112	15	17.822±2.916
	i.n.	0.52±0.33	24±8.216	17.176±8.447	1.921±0.56	30	88.45±15.311
physical mixture	i.v.	1.885±0.253	2	112.038±11.305	1.178±0.219	15	53.142±6.115
	i.n.	0.291±0.059	60	22.973±3.511	3.31±0.604	30	126.865±14.746
phospholipids complex	i.v.	1.977±0.278	2	135.352±16.292	1.782±0.318	15	60.523±6.545
	i.n.	0.605±0.051	60	59.321±3.118	4.472±0.925	30	234.351±32.104

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).

Tab.4. Influences of baicalin–phospholipid complexes on the evaluation of neurologic function

postinjury of cerebral ischemia in rats

groups	Neurologic score
sham operation group	0.00±0.00
model group	2.47±0.82
mannitol group	1.89±0.73
baicalin (i.v.)	1.93±0.67
baicalin (i.n.)	1.75±0.81
baicalin–phospholipid complexes (i.v.)	1.78±0.78
baicalin–phospholipid complexes(i.n.)	1.66±0.68
physical mixture (i.v.)	1.90±0.89
physical mixture(i.n.)	1.75±0.87

Statistical difference was calculated using Student's t-test. P<0.05.

Tab.5. Effect of drugs on brain water after focal ischemia-reperfusion injury induced by middle

cerebral artery occlusion (MCAO) in rats.(n=8, $\bar{X} \pm s$)

Groups	Water contents of brain tissues(%)
sham operation group	78.31±0.39
model group	85.64±0.85
mannitol group	79.23±0.37
baicalin (i.v.)	84.46±0.48
baicalin (i.n.)	82.32±0.77
baicalin–phospholipid complexes (i.v.)	83.48±0.76
baicalin–phospholipid complexes(i.n.)	81.10±0.65
physical mixture (i.v.)	84.27±0.89
physical mixture(i.n.)	82.21±0.60

Statistical difference was calculated using Student's t-test. P<0.05.

3.6 Pharmacological evaluation of BP complex against focal cerebral ischemia-reperfusion (I/R) injury

The neurologic deficit scores are shown in Table 4. The average score of the untreated cerebral ischemia model group was 2.47 ± 0.82 , which is significantly higher than the sham operation group. This indicated that rats in the cerebral ischemia model group did indeed have neurologic deficits. The average score of group 6 (BP complex administered intranasally) was only 1.66 ± 0.68 , significantly lower than the cerebral ischemia model group ($P < 0.01$). Thus, BP complex can delay or suppress the development of neurological signs following ischemia. Significant differences in neurologic deficit scores were also found between groups 4 (intranasally baicalin), 5, 8, and the sham operated group ($P < 0.05$). In addition, the scores of groups 3 and 7 were smaller than the cerebral ischemia model group, but the neurological deficit score was not significantly reduced.

The brain water content of each group is presented in Table 5. The brain water content of the cerebral ischemia group was significantly higher than the sham operated group, again demonstrating that the vessel occlusion model did induce significant neurological damage. The brain water content of group 6 (intranasal BP complex) was significantly lower than the untreated cerebral ischemia group ($P < 0.05$). In addition, the brain water content of group 6 was significantly lower than group 4 (intranasal baicalin), underscoring the greater efficacy of the phospholipid complex over baicalin in protecting mice against focal cerebral ischemia-induced brain edema.

The result shows that intranasal administration possesses the superiority on treating with the brain edema and cerebral ischemic damage. And for the BP complex, the reason for the improvement of the effect could be which the phospholipids increase the permeability of baicalin, on the other hand, the phospholipids can also make the therapeutic effect on healing the brain diseases.

4 Conclusion

Our present work might provide some new insights for the enhancing therapeutic effect of the brain diseases through increasing drug's concentration in brain tissue. The present investigation illustrated the use of phospholipid complex could enhance the solubility and nasal mucosa absorption of baicalin. The study also indicated that after intranasal administration, the drug could be transported to the brain directly via the olfactory mucosa. The results of the pharmacological evaluation shows when the BP complex being administered nasally, the therapeutic effect against focal cerebral ischemia-reperfusion injury has been increased significantly.

Acknowledgements

The authors are grateful for the financial support from the National Science and Technology Major Project (PR China) with project number 2009ZX09103-353.

References

[1] J. Duarte, A. Zarzuelo, J. Jimenez, J. Tanargo. Vasodilator effects of quercetin in isolated rat vascular smooth muscle. *J. Eur J Pharm* 239:1-7 (1993) .

- [2]Z.H.Gao, K.X.Huang, X.L.Yang, H.B.Xu. Free-radical scavenging and antioxidant activities of flavonoids extracted from theradix of Scutellaria-Baicalensis Geo, *J.Biochim Biophys Acta*1472:643-650(1999).
- [3]M.Bialecka.The Effect of bioflavonoids and lecithin on the course of experimental atherosclerosis in rabbits, *J.Annu Acad Med Stein* 41- 43(1997).
- [4]Z.J.Zhang,Z. Wang,X.Y.Zhang,K.Ying,J.X.Liu,Y.Y. Wang.Gene expression profile induced by oral administration of baicalin and gardenin after focal brain ischemia in rats,*J.Acta Pharmacol Sin* 26: 307-314 (2005) .
- [5]H.Lee, L.L.Yang, C.C.Wang, S.Y.Hu, S.F.Chang, Y.H.Lee.Differential effects of natural polyphenols on neuronal survival in primary cultured central neurons against glutamate-and glucose deprivation-induced neuronal death, *J.Brain.Res* 986:103-113(2003).
- [6]S.A.Elkheshen,O.A.Sammour,M.H.Shaboury,B.T.Quadeib.Ehancement of dissolution and bioavailability of piroxicam via solid dispersion with phospholipids,*J.Faculty Pharm* 39:309–320(2001).
- [7]E.W.Ulrika Olfactory transfer of analgesic drugs after nasal administration. Sweden :Uppsala University (2007)
- [8]I.J.Gill L.Ilium, N.Farraj. Cyclodextrins as protection agents against enhancer damage in nasal delivery system: Assessment of effect by measurement of erythrocyte haemolysis,*J. EurJ Pharm*1:229-236(1994).
- [9]L.Illum.Nasal durg delivery-possibilities,problems and solutions,*J.Control Release*.87:187-198(2003).
- [10]E.Martin, J.C.Verhoef, S.G.Romeijn.Effects of absorption enhance on rat nasal epithelium in vivo: release of marker compounds in the nasal cavity,*J.Pharm Res*12:1151-1157(1995).
- [11]E.Z.Longa, P.R.Weinstein, S.Carlson.Reversible middle cerebral artery occlusion without craniectomy in rats,*J.Stroke* 20:84—91(1989).
- [12]J.T.Hoff, G.Xi.Brain edema from intracerebral hemorrhage,*J.Acta Neurochir Suppl* 86:11-15(2005).
- [13]K.K.Kandimalla, M.D.Donovan.Transport of hydroxyzine and triprolidine across bovine olfactory mucosa: Role of passive diffusion in the direct nose-to-brain uptake of small molecules,*J.Int J Pharm* 302:133-144 (2003).
- [14]H.Li, H.Wang, J.H.Chen, L.H.Wang, H.S.Zhang, Y.Fan.Determination of amino acid neurotransmitters in cerebral codex of rats administered with baicalin prior to cerebral ischemia by capillary electrophoresis-laser-induced fluorescence detection,*J.Chromatogr B* 788: 93-101 (2003) .