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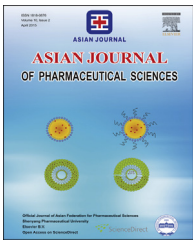

Original Research Paper

Development and evaluation of vinpocetine inclusion complex for brain targeting

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ABSTRACT

The objective of this paper is to prepare vinpocetine (VIN) inclusion complex and evaluate its brain targeting effect after intranasal administration. In the present study, VIN inclusion complex was prepared in order to increase its solubility. Stability constant (Kc) was used for host selection. Factors influencing properties of the inclusion complex was investigated. Formation of the inclusion complex was identified by solubility study and DSC analysis. The brain targeting effect of the complex after intranasal administration was studied in rats. It was demonstrated that properties of the inclusion complex was mainly influenced by cyclodextrin type, organic acids type, system pH and host/guest molar ratio. Multiple component complexes can be formed by the addition of citric acid, with solubility improved for more than 23 times. Furthermore, *In vivo* study revealed that after intranasal administration, the absolute bioavailability of vinpocetine inclusion complex was 88%. Compared with intravenous injection, significant brain targeting effect was achieved after intranasal delivery, with brain targeting index 1.67. In conclusion, by intranasal administration of VIN inclusion complex, a fast onset of action and good brain targeting effect can be achieved. Intranasal route is a promising approach for the treatment of CNS diseases.

1. Introduction

Along with the acceleration of population aging, how to treat cerebrovascular diseases effectively is a great challenge. The blood brain barrier (BBB) represents an insurmountable obstacle for a large number of drugs and is the major bottleneck in drug delivery to the brain [1,2]. So far, lots of attempts have been made to overcome the BBB, including the usage of carrier-mediated transporters (CMT), receptor-mediated transporters (RMT), and nano-sized systems such as nanosuspension, nanoparticles and micelles with different administration routes [3]. Among them, intranasal drug delivery is one of the focused delivery options for brain targeting. As a kind of non-invasive route, intranasal drug delivery has the advantages of rapid onset of action, good patient compliance, and avoiding hepatic first pass effect with high bioavailability [4]. Moreover, the brain and nose compartments are connected to each other

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via the olfactory receptor cells, they are the only surface neural cells of the body, so the olfactory mucosa could be considered as a 'window to the brain' [5], thus the BBB can be passed by and a wide variety of therapeutic agents, including both small molecules and macromolecules, can be successfully delivered to the CNS by intranasal route [6].

Vinpocetine (VIN) is a vasoactive vinca alkaloid and a synthetic derivative of apovincamine, it is commonly used in clinical practice for the treatment of disorders arising from cerebrovascular and cerebral degenerative diseases [7,8]. However, due to its poor solubility in water [9], short elimination half-life (1–2 h) and extensive metabolism in liver (~75%) [10,11], it has low oral bioavailability (6.7%) in human beings [12] and therefore low drug concentration in the brain, limiting its application in the clinic. Therefore, it is highly desirable to design appropriate drug delivery system for VIN with improved drug solubility and enhanced brain targeting effect.

Different methods can be used to increase the solubility of poorly soluble drugs. Among them, inclusion complex formation is an effective one. Cyclodextrin (CD) and its derivatives, which can form "inclusion complexes" in aqueous solutions, have been widely used in pharmaceutics to increase the solubility, stability and bioavailability of poorly soluble drugs, with reduced irritation and side effects [13].

Thus, the objective of this study is to improve the solubility of VIN by inclusion complex formation and increase its brain targeting effect by intranasal administration. Solubility phase diagram was used to guide the preparation of the inclusion complex. The complexes formation was confirmed by DSC and solubility test. Influence of different factors on the properties of the complex was investigated. Brain targeting effect of VIN inclusion complex was evaluated after intranasal administration in rats.

2. Materials and methods

2.1. Materials

Vinpocetine (VIN) was purchased from Haide Corporation (Benxi, Liaoning, China). β-cyclodextrin (β-CD) was from Tianjin Chemical Reagent Company (Tianjin, China), hydroxypropyl-β-cyclodextrin (HP-β-CD) was from Deli Biological Chemical Corporation (Xi'an, Shanxi, China), randomly methyl-β-cyclodextrin (RM-β-CD) was from Xinda Chemical Corporation (Jinan, Shandong, China), citric acid was from Zhengxin Institute Department of reagents (Shenyang, Liaoning, China), tartaric acid was from Bodi Chemical Company (Tianjin, CA), Methanol (HPLC grade) was supplied by Yuwang (Jinan, Shandong, China). All other reagents and buffer components were of analytical grade.

2.2. Solubility test

The solubility of VIN under different conditions was measured using shake-flask method at 37 °C [14]. Briefly, an excess amount of VIN was added to 5 ml of specific solvents and the samples were placed in a water bath and stirred at 100 r/min for 48 h. Thereafter, the resulting suspensions were filtered through a 0.45 μm membrane filter and concentration of VIN in the filtrate was analyzed using high performance liquid chromatography (HPLC) method after dilution. The HPLC instrument consists of Agilent C18 column (4.6 mm*150 mm, 5 μm, USA) and UV detector set at 273 nm. Mobile phase was a mixture of methanol: ammonium acetate (15.4 g/l) (80:20, v/v), filtered through 0.45 μm membrane filter, the flow rate was 1.0 ml/min, injection volume 20 μl, oven temperature 35 °C.

2.3. Phase solubility studies

The phase solubility study of VIN with β-CD (0–12.5 mM), HP-β-CD (0–90.0 mM) and RM-β-CD (0–77.0 mM) was performed at 37 °C in distilled water (pH = 6.3). The stability constant (Kc) of the complex was calculated according to the following equation [15]:

$$Kc = \text{slope}/\text{intercept}(1 - \text{slope})$$

The higher the Kc value, the better the stability.

2.4. Preparation of inclusion complex

The inclusion complex was prepared by dissolving followed freeze-drying method. Briefly, 1400 mg of HP-β-CD was dissolved in 10 ml of distilled water at room temperature and a solution of 10 ml 2% (w/v) citric acid or tartaric acid, aqueous solution containing appropriate amount of vinpocetine (the molar ratio of VIN and HP-β-CD was 1:1, 1:2, 1:3) was added under stirring, ultrasounded for 15 min to dissolve the drug and HP-β-CD. The resulting solution was stirred at 50°Cfor 1–2 h. After equilibrium to room temperature, pH of the solution was adjust to approximately 5 unless specially indicated, and filtered through 0.45 μm membrane filter, the clear solution was frozen at −20 °C and subsequently freeze-dried (FD-1 freeze-dryer apparatus, Beijing Medicine and Health Technology Co, Beijing, China) for 48 h. The inclusion rate is calculated according to the following equation:

$$\text{The inclusion rate} = \frac{\text{weight of VIN in inclusion complex}}{\text{weight of VIN fed initially}}$$

2.5. Characterization of inclusion complexes

Formation of inclusion complexes was identified by solubility study as described in 2.2 and DSC analysis. The DSC curves were determined with a DSC instrument (DSC-1, METTLER, Switzerland) under the following conditions: samples (2–3 mg) were hermetically sealed in a flat-bottomed aluminum pan and heated, with an empty pan sealed as reference, over a temperature range of 20–250 °C with the heating rate of 10 °C under nitrogen gas.

2.6. In vivo analytical method of VIN

VIN concentration in plasma was analyzed by HPLC after solvent extraction [17]. Briefly, 200 μl of the plasma sample was mixed with 40 μL of internal standard (8 μg/ml progesterone) and 50 μL of 0.5 M NaOH solution for 30 s by vortexing in a glass tube. Then hexane (3 ml) was added for VIN extraction. The mixture was centrifugalized and the supernatant was transferred into another glass tube and the solvent

was evaporated in a 40 °C water bath under nitrogen flow. The residue was redissolved in 100 μl of methanol by vortexing, followed by centrifugation at 6650 g/min for 10 min. The supernatant was analyzed by HPLC at wavelength 228 nm to increase the sensitivity; the other analytical conditions were the same as described in Section 2.2. For the brain homogenate, 0.5 ml of homogenate was mixed with 40 μl of internal standard and 50 μl of 0.5 M NaOH solution by vortexing for 30 s in a glass tube, hexane (6 ml) was added for extraction, and then follow the procedures described above.

The HPLC method described above for VIN assay in the plasma and in the brain tissue was specific and efficient. There was no interference from endogenous components observed at the retention time of the analytes in the chromatogram. Good linear relationship between drug concentration and peak area was established, the concentration range was 20–1000 ng/ml for plasma sample and 20–800 ng/ml for brain samples. The mean extraction recoveries were 90.18% and 85.39% for plasma and brain samples, respectively. The RSDs of intra- and inter-day precision were both below 10.0%.

2.7. Pharmacokinetic studies in rats

In vivo absorption of VIN inclusion complex after intravenous and intranasal administration was studied in rats. All animal studies were approved by the University Ethics Committee and were carried out in accordance with the Principle of Laboratory Animal Care. The Sprague Dawley rats (male, 180–200 g) were supplied by the Lab Animal Center of Shenyang Pharmaceutical University and randomly divided into two groups, 15 rats in each group. In group I the complex was given by intravenous injection via tail vein, in group II the complex was administrated intranasally according to the method reported previously [16], both with a single dose of 1 mg/kg. Blood samples (approximately 0.5 ml) were withdrawn from retro-orbital plexus at 0, 3, 5, 10, 20, 30 min, 1, 2, 3 h post-dosing ($n = 3$) and centrifuged at 6650 g/min for 10 min. The supernatant plasma samples were stored at −20 °C until analysis.

For the biodistribution study, the rats were sacrificed at 10 min, 30 min, 1 h, 2 h after dosing ($n = 3$) and the brain was removed after cardiac perfusion with 0.9% NaCl solution, and drug concentration in the brain was determined by homogenizing the organs in 2-fold ice-cold 0.9% NaCl solution before analysis.

2.8. Statistical analysis

Pharmacokinetic parameters, the maximum plasma concentration of the drug (C_{max}), the time to reach C_{max} (T_{max}) and AUC were calculated from the plasma concentration–time profiles. The absolute bioavailability (F) of the intranasally administrated inclusion complex was calculated by comparing their AUC with that of intravenous injection using DAS 2.1.1 software. Significance of difference was evaluated using one-way ANOVA at the probability level of 0.05. Drug targeting index (DTI) was calculated by the following formula, DTI >1 was considered as brain-targeting distribution [18].

$$DTI = \frac{(AUC_{brain}/AUC_{plasma})_{i.n}}{(AUC_{brain}/AUC_{plasma})_{i.v}}$$

3. Results and discussion

3.1. Influence of pH on the solubility of VIN

To optimize the condition for inclusion complex preparation, influence of pH on the solubility of VIN was studied firstly. The pH range was selected based on the physiological condition of human body fluid and nasal mucus [19]. Buffer solutions with pH 2.0, 4.0, 5.0, 6.0, 6.8, 7.4 were used in this study.

As shown in Table 1, with the increase of pH, the solubility of VIN decreased significantly. The solubility of VIN was 5.43 mg/ml at pH 2, and decreased to 2.44 μg/ml at pH 6.8. As VIN is a kind of weak basic drug (pKa 7.31), its solubility was greatly affected by pH, therefore it is absolutely essential to monitor pH during the experiment.

3.2. Influence of cyclodextrin type on the stability of the inclusion complex

To screen which kind of cyclodextrin can form stable complex with VIN and explore the complexation ratio, phase solubility diagrams of VIN with three types of cyclodextrin, including β-CD, and two water soluble cyclodextrin derivatives, 2-hydroxypropyl derivatives of β-cyclodextrin (HP-β-CD) and randomly methyl-β-cyclodextrin (RM-β-CD) were studied in distilled water (pH 6.3). As shown in Fig. 1, irrespective of cyclodextrin type, a typical A_L type diagram was observed and the solubility of VIN increased almost linearly with the increase of CD concentration, implying that soluble VIN and CD inclusion complexes were formed at molar ratio 1:1.

Meanwhile, the apparent stability constant (K_C) of different complexes, which represents strength of the interaction and stability of the complex, were calculated, it was in the order: HP-β-CD (77.77 M^{-1}) > RM-β-CD (74.76 M^{-1}) > β-CD (66.92 M^{-1}). No significant difference in bonding force between VIN and HP-β-CD, RM-β-CD were found, both are higher than β-CD based complex. Considering the fact that HP-β-CD has better safety profile for intranasal administration than that of RM-β-CD [20], and the poor aqueous solubility of β-CD (1.85% at 25 °C), HP-β-CD was selected for further investigation.

However, even when the concentration of HP-β-CD was as high as 15%, the solubility of VIN was just 50–60 μg/ml, far less than the dose required for intranasal delivery. It is reported that multicomponent complexation technology is quite effective in increasing drug concentration in the cavity of

Table 1 – Influence of pH on the solubility of VIN at 37 °C ($n = 3$).

pH	2	3	4	5	6	6.8	7.4
Solubility C (mg/ml)	5.43	1.87	0.51	0.093	0.012	$2.44*10^{-3}$	$7.8*10^{-4}$

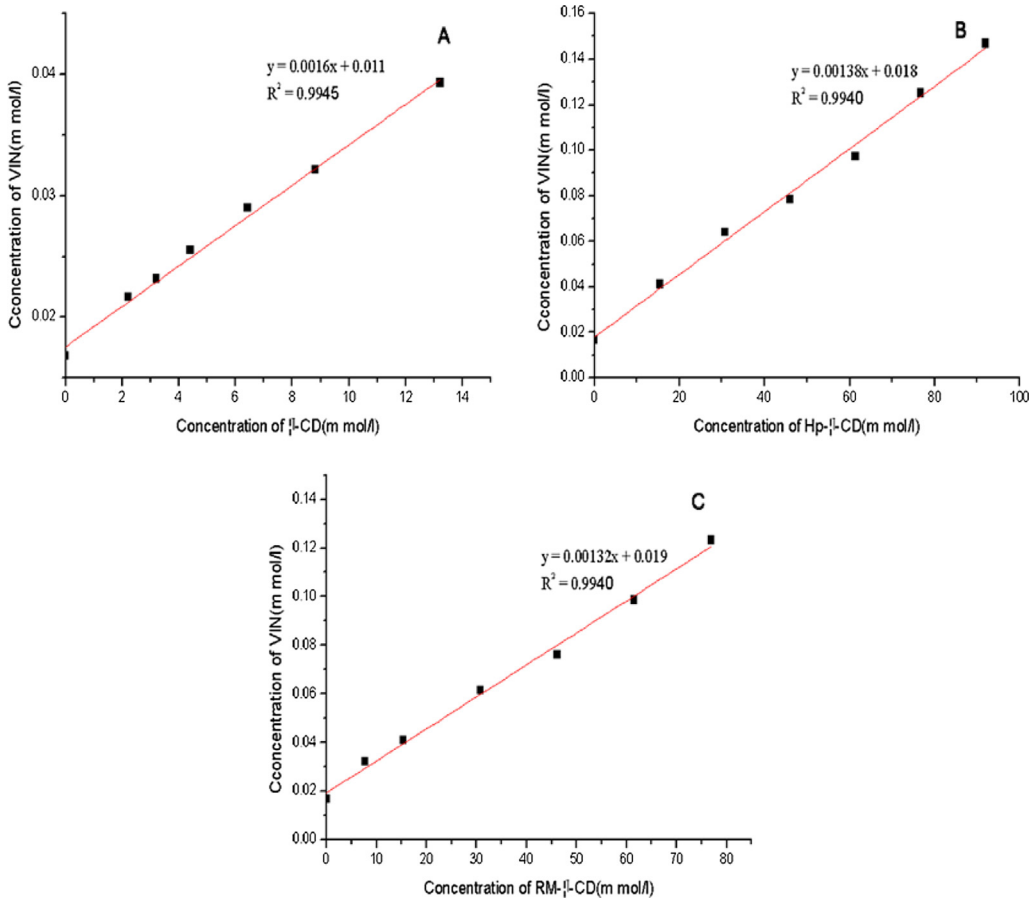

Fig. 1 – Phase solubility diagrams of VIN with different kinds of β-CD in distilled water (pH = 6.3) and 37 °C (A:β-CD, B: HP-β-CD, C: RM-β-CD).

cyclodextrin, the perquisite for using this technology is that a tight fit between the complexed molecule and the CD must occur (Kc at least 10^3 M^{-1}) [21], apparently, the interaction between VIN and cyclodextrin was far below this value. Fortunately, it was found that citric acid (CA) can increase the solubility of VIN significantly [22], and the solubility enhancement of CA overweighed the influence of solution pH. For example, the solubility of VIN in 2% CA was 15 mg/ml (the pH of different concentrated CA solution was typically in the range of pH 3–4), and in comparison, its solubility in pH 3.0 medium was only 1.87 mg/ml, implying free water soluble complexes were formed between VIN and CA [22]. Therefore, adding CA might be an effective way to improve VIN concentration in the HP-β-CD by forming multicomponent complex. It has been reported that simultaneous complexation with these acids significantly increased the solubilizing power, reducing the amount of cyclodextrin necessary for making the targeted formulation [21].

3.3. *Factors influencing properties of VIN inclusion complex*

To optimize properties of VIN inclusion complex, influence of VIN and HP-β-CD molar ratio (1:1, 1:2, 1:3), organic acids type (citric acid and tartaric acid), inclusion complex preparation temperature (40, 50, 60 °C), inclusion incubation time (1, 2, 3 h), and the system pH (4.5, 5, 5.5) on achieved drug concentration and the inclusion rate were investigated. The results are presented in Table 2.

First of all, by keeping solution pH at 5, influence of different parameters was investigated. Taking both drug

Table 2 – Factors influencing properties of VIN inclusion complex.

Factors		The concentration of VIN(μg/ml)	Inclusion rate (%)
Organic acid	Citric acid	4743.75	85.39
	Tartaric acid	4054.45	72.98
Molar ratio (VIN:CD)	1:1	4875.93	29.26
	1:2	4854.07	58.25
	1:3	4743.75	85.39
Temperature (°C)	40	4594.04	82.69
	50	4743.75	85.39
	60	4666.84	84.00
Time (h)	1	4730.73	85.15
	2	4743.75	85.39
	3	4437.64	79.88
System pH	4.5	5491.14	98.80
	5	4743.75	85.39
	5.5	363.31	6.54

concentration and inclusion rate into consideration, citric acid was more effective than that of tartaric acid and was selected for the followed study. No significant difference in drug concentration was found ($P > 0.05$) when the molar ratio of VIN and HP-β-CD was 1:1, 1:2, 1:3, but the inclusion rate increased with the increase of molar ratio, so VIN and HP-β-CD molar ratio 1:3 was selected. Influence of temperature on properties of the inclusion complex was not significant in the range of 40–60 °C, 50 °C was selected. When the incubation time was 1 h or 2 h, there was no significant difference in drug concentration or inclusion rate, however, when the reaction time was prolonged to 3 h, the drug concentration decreased slightly, therefore reaction time 1–2 h was selected. As to the influence of system pH, drug concentration decreased approximately 14% when the pH was changed from 4.5 to 5, however, a sharp decrease in drug concentration was found when the pH was adjusted to 5.5, probably the organic acid was neutralized by the added base and destroyed the multiclathrate complex [21]. Since pH 4.5–6.5 is commonly accepted for intranasal administration [23], pH 4.5 was selected for the followed study.

Therefore, the final complex was prepared by using the following conditions: VIN and HP-β-CD molar ratio 1:3, organic acid types: citric acid, inclusion temperature 50 °C, incubation time 1–2 h, the system pH 4.5.

3.4. *Identification of inclusion complex formation*

Formation of inclusion complexes was identified by both DSC and solubility study. The DSC profiles of the pure drug, the ternary system of the multicomponent complex and physical mixture are shown in Fig. 2. The thermal curve of pure VIN was typical of a crystalline anhydrous substance with a sharp endothermic peak at 151.98 °C corresponding to its melting point [24]. The melting peak was also observed in the physical mixture. Concerning the ternary system of the multicomponent complex, the thermal characteristic peak of VIN was shifted to a lower temperature at around 148.0 °C and its intensity decreased significantly, indicating that most of drug substance existed in amorphous state and/or being included in the complex [24]. These results suggest that VIN/HP-β-CD/CA inclusion complexes were formed.

Inclusion complex formation was further confirmed by solubility test. The drug solubility of the inclusion complex lyophilized powder was 4.74 mg/ml (pH = 4.5), and it was 0.2 mg/ml for the free drug measured at exactly the same condition. This study indicated that the solubility of VIN in the inclusion complex was about 23-fold higher than that of the free drug in water.

3.5. *Pharmacokinetic and biodistribution studies*

The concentration–time profiles of VIN in the plasma and in the brain after i.v and intranasal administration of VIN inclusion complex in rats are shown in Fig. 3. The pharmacokinetic parameters are summarized in Table 3. The plasma AUC value of VIN after intranasal administration was slightly lower than that after i.v. administration, and the absolute bioavailability was 88%, with T_{max} 10 min, implying that VIN can be quickly absorbed into systemic circulation comparable with that of intravenous injection.

As for brain targeting, shorter T_{max} and higher drug concentration in the brain was found after intranasal administration of VIN complex. The mean C_{max} values in the brain (153.5 ng/g) after i.n administration were found to be markedly higher than those obtained after i.v administration (78.5 ng/g). The peak concentration in the brain occurred at 10 min after i.n administration, and it was faster than that of i.v (30 min) route. Moreover, the brain $AUC_{0-180\ min}$ value was significantly higher after intranasal administration compared to i.v. administration, with DTI value 1.67, indicating significant brain-targeting effect was achieved. This is quite reasonable and can be explained by the fact that part of the drug could reach the brain directly via olfactory mucosa in the nose whereas after i.v administration, the drug will be firstly absorbed into systemic circulation then redistributed in the

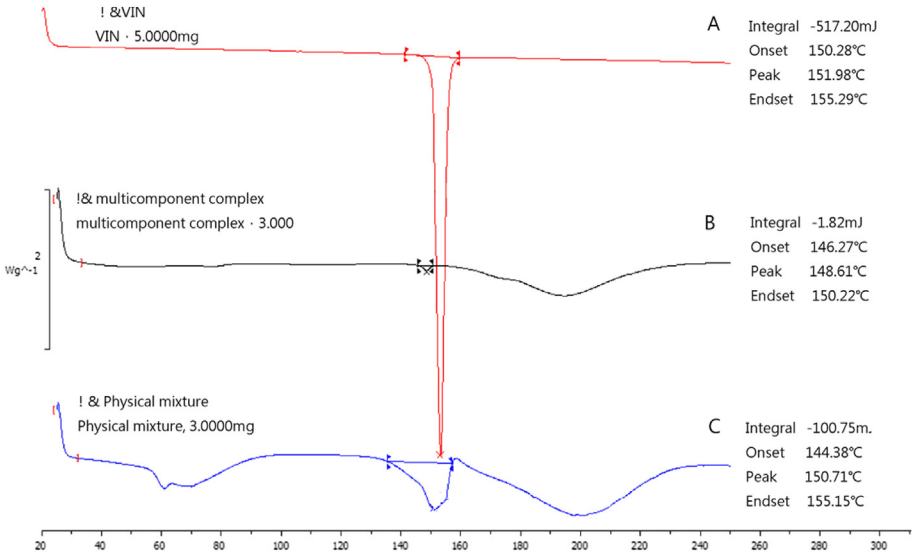

Fig. 2 – DSC curves of VIN, VIN/HP-β-CD/CA inclusion complex and their physical mixture (A: VIN, B: VIN/HP-β-CD/CA inclusion complex,C: physical mixture).

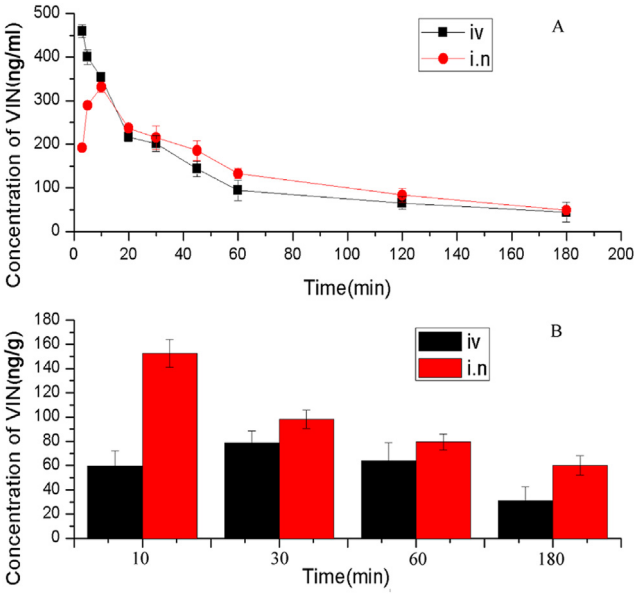

Fig. 3 – Concentration–time profiles of VIN in the plasma and in the brain after intranasal and intravenous injection in rats ($n = 3$) (A: plasma, B: brain).

Table 3 – The pharmacokinetic parameters in plasma and brain after intravenous (i.v) and intranasal (i.n) administration of VIN inclusion complex in rats ($n = 3$).

Parameters	i.v	i.n
$(AUC_{0-t})_{plasma}$(ng·min/ml)	22,391.0 ± 1169.7	19,700.4 ± 1288.4*
$(AUC_{0-t})_{brain}$(ng·min/g)	9229 ± 1615.0	13,544 ± 1012.0**
$(C_{max})_{plasma}$ (ng/ml)	459.4 ± 14.7	301.0 ± 9.1**
$(C_{max})_{brain}$(ng/g)	78.5 ± 11.5	153.5 ± 16.2**
$(T_{max})_{plasma}$(min)	2 ± 0	10 ± 0**
$(T_{max})_{brain}$(min)	30 ± 11.54	10 ± 0**

*P < 0.05, compared to the i.v administration.
**P < 0.01, compared to the i.v administration.

brain slowly [25]. This is in good agreement with the higher drug concentration in plasma than that in brain after i.v. injection.

4. Conclusions

In the present study, VIN inclusion complex was prepared in order to increase its solubility. Factors influencing properties of the inclusion complex was investigated. Formation of the inclusion complex was identified by solubility study and DSC analysis. *In vivo* study in rats revealed that after intranasal administration of vinpocetine inclusion complex, the absolute bioavailability was 88%. Significant brain targeting effect was found with intranasal administration of VIN compared with that of intravenous injection, with brain targeting index 1.67. In conclusion, by intranasal administration of VIN inclusion complex, a fast onset of action and good brain targeting effect can be achieved.

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