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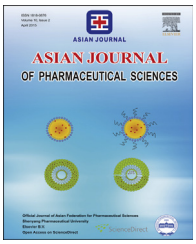

Original Research Paper

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