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ScienceDirect
International Journal of Pharmaceutics 337 (2007) 127–132
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Huperzine A in rat plasma and CSF following intranasal administration

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Received 11 October 2006; received in revised form 14 December 2006; accepted 22 December 2006
Available online 28 December 2006

Abstract

This paper presents to investigate the levels of Huperzine A in plasma and CSF of rats after three different kinds of administrations and to find out whether intranasal administration is the best root to transfer the drug into the CNS. The drugs of two doses (167 and 500 μg/kg) were administered to male Sprague–Dawley rats intravenously, intranasally and intragastricly, respectively. Series plasma and cerebrospinal fluid (CSF) samples were collected from femoral artery and cisterna magna for 6 h. The drug concentrations were determined by HPLC-fluorescence method. The AUC_{plasma} and the AUC_{CSF} of intranasal administration were 90.3% and 127.7% in low dose group (167 μg/kg) and 91.3% and 69.4% in high dose group (500 μg/kg) compared with intravenous administration. The AUC_{plasma} and the AUC_{CSF} of intragastric administration were 98.9% and 52.1% in high dose group (500 μg/kg) compared with intravenous administration.

Keywords: Huperzine A; Plasma; CSF; Intranasal administration

1. Introduction

In recent decades, some few bio-active ingredients and promising compounds have been discovered from traditional Chinese medicinal plants. Among them Huperzine A is obviously a famous one (Bai et al., 2000; Jiang et al., 2003). Pharmacological studies demonstrated that Huperzine A is a potent selective and reversible inhibitor of AChE, and showed memory-enhancing effects on behavioral models in animals. It was used to treat myasthenia gravis before, but now it was proved to be clinically useful as a palliative agent for Alzheimer's disease in China and was already marketed in USA as a dietary supplement.

In order to develop an optimized dosage form and to find a proper administration route for this drug, we compared three dosage forms following three kinds of administration route, hoping to discover the best way which could deliver more drugs directly into the brain meanwhile minimize the peripheral cholinergic side effects of Huperzine A. By a simple method we could obtain serial cerebrospinal fluid (CSF) samples for up to 6 h (Patsalos et al., 1992; Lolin et al., 1994). From the CSF concentrations we could know how much the drug was delivered into the brain.

Recently many researches have focused on the intranasal drug delivery system, and the results indicated that many compound involving with antihistamines, l-DOPA, local anesthetics, dihydroergotamine, (s)-UH-301, physostgmine analogue, dopamine, benzoylecgonine, cocain, indomethacin, stavudine, ^{11}C-zolmitripatan et al. could be transferred into the central nervous system (CNS) through the nasal route (Chou and Donovan, 1997, 1998; Kao et al., 2000; Wang et al., 1998; Dahlin and Bjork, 2000, 2001a; Dahlin et al., 2001b; Chow et al., 1999, 2001; Yang et al., 2005; Dontas et al., 2004; Yates et al., 2005). A relatively large surface area, and a thin, porous and vascularized epithelium with plenty blood supplement ensure rapid absorption and onset of therapeutic agent after intranasal administration (Mygind and Dahl, 1998; Behl et al., 1998). But what attracted us was its property to deliver drugs into CNS directly (Illum, 2000; Ugwoke et al., 2001). In the last 20 years, more and more attentions were paid here. With the natural structure close to the brain, the olfactory region has three possible pathways to deliver drugs into brain: transcellularly, paracelularly, and olfactory nervous pathway. And some simple principles have been obtained, for example if the drug is lipophilic and has a small molecule weight, it would have a good permeability into the CNS. And Huperzine A is supposed to be such a drug which is a fat-soluble weak alkaloid with a molecule weight of 242.32, but whether it could be directly delivered into the CNS through the nasal way still need investigation in this research.

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doi:10.1016/j.ijpharm.2006.12.029

2. Materials and methods

2.1. Chemicals

Huperzine A was provided by Wan Bang Pharmaceutical Co. (Zhejiang, China). Its purity was 99.6%, verified by Zhejiang Provincial Institute for Drug Control. Huperzine A intranasal spray (2 mg/ml) was developed by Pharmaceutical Department of Shanghai Institute of Pharmaceutical Industry. The Huperzine A tablets (50 μg/tablet) were purchased from the market.

Borax, sodium carbonate, and triethanol amine were analytical grade. Chloroform, and methanol were HPLC grade. Distilled water, prepared from demineralized water, was used throughout the study. SD rats were obtained from experimental animal center of Fudan University.

2.2. Animal preparation

The animal experiment was carried out in compliance with the protocol of Animal Use and Care by Medical Center of Shanghai Institute of Pharmaceutical Industry. Male Sprague–Dawley (300–350 g) rats were housed with water and standard laboratory food given ad libitum. The animals were anesthetized with an intraperitoneal dose of 30% (w/v) urethane (2 g/kg) which had shown no inhibitory effect on either retrograde and anterograde axoplasmic transport and was proved to be superior to other anesthetics in this study. The animals were kept under anesthesia throughout the experiment. CSF samples were collected from cannulation in cisterna magna with some fine adjustment (Huang et al., 1995; Berg et al., 2002). Briefly: when the anesthetized animal was fixed onto the stereotaxic apparatus (Jiangwan I-C, Shanghai, China), the skin overlaying the occipital bone was cut and then the underlying muscles and tissues were bluntly separated so that the atlanto-occipital membrane was identified and freed from other tissues. A 25 gauge needle connected to a 10 cm PE-10 tube was punctured into the cisterna magna through the a–o membrane, once the CSF flowed into the tube under the inner pressure the mucilage was then used to fix the needle with the membrane. A 100 μl microsyringe was connected to the other end of the PE-10 tube, and a 20 μl volume of CSF was withdrawn at each sampling point. After that the femoral artery and vein were cannulated with two pieces of 5 cm PE-50 tubes, the former was for blood sampling and the latter was for body fluid replenishing. Ten milligrams per killogram heparin sodium salt was administered soon after cannulation for whole body anticoagulation. A 500 μl volume of blood was obtained at each sampling point and every blood sample was replaced with the same volume of physiological saline for body fluid replenishing.

2.2.1. Drug preparation

Huperzine A was directly dissolved in physiological saline to form a solution of 50 μg/ml for intravenous administration. For intranasal administration, the nasal gel was prepared as following: first, 5% (w/v) mannitol, 0.18% (w/v) methyl parahydroxybenzoate, 0.02% (w/v) ethyl parahydroxybenzoate, and 0.5% (w/v) gellan gum were dissolved in demineralized water of volume 85% after heating. Second, Huperzine A was dissolved in 0.1 mol/l hydrochloric acid of volume 10%. Third, the two solutions mentioned above were mixed together and added with water to volume 100% after adjusted to pH 6.5 with trihydroxymethyl aminomethane. Finally the Huperzine A nasal gel of 2 mg/ml was got.

2.2.2. Intravenous administration

For intravenous administration, 167 and 500 μg/kg doses were administered to rats via femoral vein. Blood and CSF samples were collected at predetermined sampling points (2, 5, 15, 30, 45, 60, 120, 180, 240, 360 min).

2.2.3. Intranasal administration

For intranasal administration, 167 and 500 μg/kg doses were administered to rats. A volume of 25 μl (for 167 μg/kg dose) or 75 μl (for 500 μg/kg dose) nasal gel was placed into one nostril by carefully inserting a 2 cm length of PE-10 tube attached to a 100 μl microsyringe. Blood and CSF samples were collected at predetermined sampling points (5, 15, 30, 45, 60, 90, 120, 180, 240, 360 min).

2.2.4. Intragastric administration

For intragastric administration, only 500 μg/kg dose was administered to rats (dose 167 μg/kg was undetectable in CSF). Before administration, the esophagus was isolated and inserted with a 27 gauge needle connected with a very short piece of PE-50 tube for avoiding hurting the esophagus. Then three Huperzine A tablets (50 μg/tablet) were dissolved in 2 ml water and administered through the needle. Blood and CSF samples were collected at predetermined sampling points as the same as intranasal administration.

2.3. Analytical procedures

The CSF and blood samples were analyzed by the HPLC-fluorescence method with different pre-disposals. CSF samples were stored at −20 °C immediately after collection without further treatment. After thawed and centrifuged at 10,000 r/min, CSF samples were analyzed directly with an injection volume of 20 μl. Blood samples were centrifuged at 3000 r/min, and the plasma was separated and stored at −20 °C. A 200 μl of rat plasma, 10 μl of the internal standard working solution and 100 μl of Borax-sodium carbonate buffer solution (pH 11.8) were added to a 10 ml conical glass centrifuge tube. After briefly vortex, the mixture was extracted with 2 ml of chloroform by vortex-mixing for 5 min. After centrifugation at 3000 r/min for 10 min, the organic phase was transferred to another clean glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 50 μl mobile phase, an aliquot of 20 μl of the resulting solution was injected into the HPLC system (Wang et al., 2004; Ye et al., 2005).

The Shimadzu HPLC system consisted of two LC-10ADVP pumps, one RF-10AXL fluorescence detector, a