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Huperzine A in rat plasma and CSF following intranasal administration

Peng Yue, Tao Tao *, Yan Zhao, Jinfeng Ren, Xuyu Chai

Division of Pharmaceutics, Shanghai Institute of Pharmaceutical Industry, ZhongShanBeiYi Road 1111, Shanghai 200437, China

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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.

* Corresponding author. Tel.: +86 21 55514600 124; fax: +86 21 65420806.
E-mail address: taotaosipi@hotmail.com (T. Tao).

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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

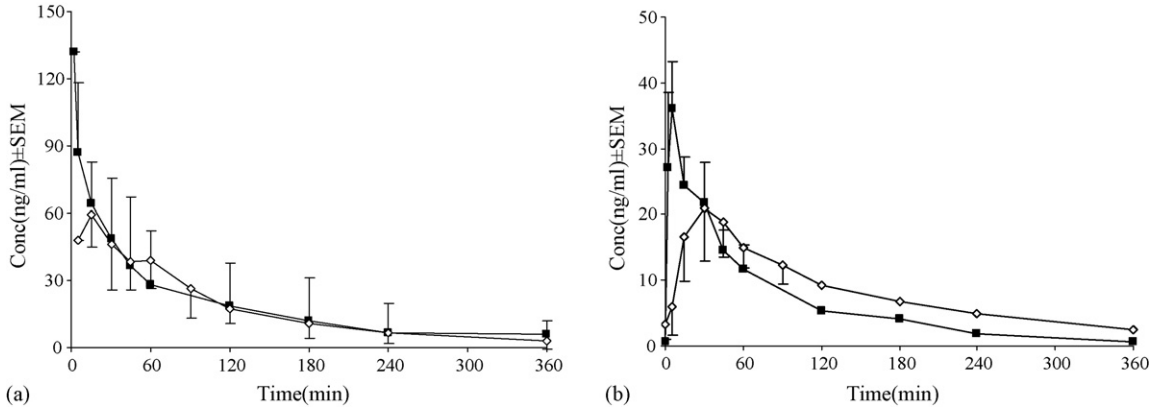

Fig. 1. Huperzine A concentrations in (a) plasma and (b) CSF following intravenous (■) and intranasal (□) administration (dose:167 μg/kg). Data represent the mean ± S.E.M. (n = 6, 6).

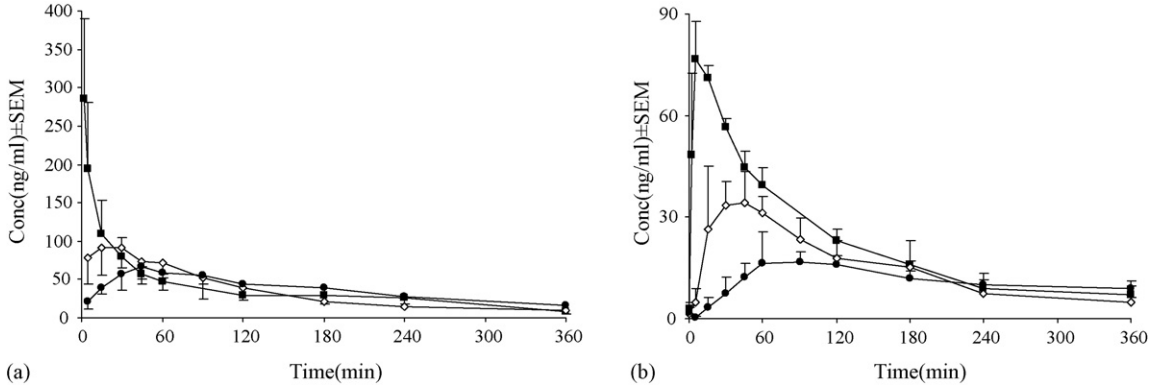

Fig. 2. Huperzine A concentrations in (a) plasma and (b) CSF following intravenous (■), intranasal (□) and intragastric (●) administration (dose: 500 μg/kg). Data represent the mean ± S.E.M. (n = 5, 6 ,5).

CTO-10AVP column oven, a SCL-10VP system controller and a CLASS-VP HPLC work station. An Elite ODS C18 (250 mm × 4.6 mm, 10 μm) analytical column and a mixture of methanol–water–triethanol amine (60:40:0.05,v/v/v) mobile phase were used for CSF determination. (Yue et al., 2005) A Kromasil ODS C8 (150 mm × 4.6 mm, 5 μm) analytical column and a mixture of methanol–water–triethanol amine (45:55:0.05,v/v/v) mobile phase were used for plasma determination. Both had a flow rate of 1.0 ml/min. Fluorescence detector had excitation at 310 nm and emission at 370 nm and the temperature of column oven was set at 40 °C.

2.4. Data analysis

Results from HPLC analyses were plotted as drug concentrations in the CSF and plasma versus time. Compartment-model pharmacokinetic parameters in CSF and plasma were described with 3p97 kinetic program. Non-compartment pharmacokinetic parameters including: T_{max}, C_{max}, $t_{1/2}$ and AUC were estimated by Kinetica 2000 computer program. The AUC values for each curve were calculated from time zero to the last data point using the trapezoidal rule without extrapolation to infinity. Statistical significance among intravenous, intranasal and intragastric administration were determined using the Student's t-test with $p < 0.05$.

3. Results

Drug concentration profiles of two doses in the plasma and CSF following intravenous, intranasal and intragastric administration are shown in Figs. 1 and 2. And in order to get information of the Huperzine A disposal in the CSF, 5 μg drugs were directly injected into the lateral compartment and the CSF

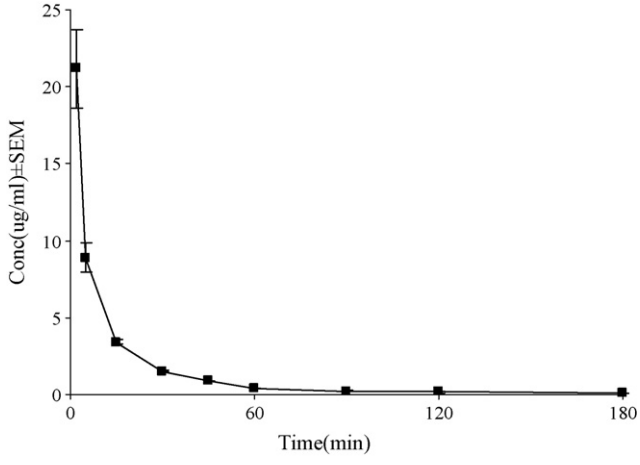

Fig. 3. Huperzine A concentrations in CSF following direct lateral compartment injection (■). Data represent the mean ± S.E.M. (n = 3).

Table 1
Non-compartment pharmacokinetic parameters of Huperzine A in blood and CSF of two doses following intravenous, intranasal and intragastric administration

Dose (μg/kg)	Blood				CSF			
	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0\rightarrow t}$ (ng h/ml)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0\rightarrow t}$ (ng h/ml)	$t_{1/2}$ (h)
167 (IV)	0.033 ± 0.0	132.3 ± 31.3	112.8 ± 78.2	1.80 ± 0.76	0.07 ± 0.03	36.9 ± 7.3	37.9 ± 10.2	1.11 ± 0.32
167 (IN)	0.26 ± 0.13	60.0 ± 19.3	101.9 ± 29.2	1.44 ± 0.61	0.63 ± 0.26	23.9 ± 5.8	48.4 ± 8.8	1.97 ± 0.66
500 (IV)	0.04 ± 0.02	285.6 ± 105.0	219.7 ± 40.0	2.03 ± 0.75	0.12 ± 0.07	78.6 ± 9.5	129.6 ± 8.9	1.71 ± 0.43
500 (IN)	0.38 ± 0.14	104.1 ± 34.3	200.6 ± 33.6	1.82 ± 0.23	0.67 ± 0.26	40.5 ± 11.0	89.9 ± 12.2	1.96 ± 0.66
500 (IG)	0.85 ± 0.38	71.6 ± 24.7	217.3 ± 82.9	2.47 ± 0.77	1.70 ± 0.45	21.2 ± 6.4	67.6 ± 4.1	3.55 ± 0.29

concentration profiles is shown in Fig. 3.The data represents the mean ± S.E.M. of the concentration measured. Pharmacokinetic parameters calculated by Kinetic 2000 program are showed in Table 1. Compartment model parameters in CSF and blood calculated by 3p97 kinetic program are showed in Table 2.

After intravenous administration of both doses the initial drug concentrations in blood were about 132 ng/ml (167 μg/ml) and 285 ng/ml (500 μg/ml), respectively. And the drug rapidly appeared in the CSF with T_{max} of about 0.07 h (167 μg/ml) and 0.12 h (500 μg/ml), respectively. The results demonstrated that Huperzine A could pass through the blood brain barrier easily by simple diffusion as supposed according to the rule of physical chemical characteristics. And the drug eliminated from both the blood ($t_{1/2} < 2.03$ h) and the CSF ($t_{1/2} < 1.71$ h) rapidly.

After intranasal administration Huperzine A was rapidly absorbed into the blood with $T_{max} < 0.38$h and C_{max} about 60 ng/ml (167 μg/ml) and 104 ng/ml (500 μg/ml). The drug concentrations in the CSF did not reach the C_{max} as fast and high as the intravenous administration with several minutes later than in the blood. However, the CSF concentrations were shown to be slightly higher than the intravenous administration after 30 min and this trend was maintained until 6 h for dose (167 μg/kg) which contributed to the ratio 1.28 of AUC_{CSF} (IN/IV). Although some parts of drugs appeared to be delivered directly into the CSF, only a very small mount of them (less than 0.11%) could achieve that, calculated by comparing the AUC_{CSF} of various administrations with the AUC_{CSF} after direct lateral compartment injection (dose 5 μg).

Although the drug had been totally dissolved before intragastric administration, it showed a much slower absorption rate both to the general circulation and to the CSF than the intranasal administration. The T_{max} of the intragastric administration in blood was 0.85 h and the T_{max} of CSF was 1.7 h. Because of the lag of absorption, drug concentrations in both blood and CSF were slightly higher than the intravenous and intranasal administration at 4 and 6 h.

And one interesting thing was found in the pharmacokinetic disposal of Huperzine A in both the blood and the CSF that it demonstrated to be a typical, two-compartment model, not only in the general circulation but in the CNS compartment. Rapid and severe side-effects were observed during the experiment especially in intravenous administration such as muscle trepidation

Table 2
Compartment model parameters (compartment number = 2, weight = 1/*C*) of Huperzine A in plasma and CSF

Parameters	Plasma[a]		CSF[a]	
	167 (μg/kg)	500 (μg/kg)	167 (μg/kg)	500 (μg/kg)
A (ng/ml)	113.72 ± 48.10	237.12 ± 113.35	34.67 ± 18.31	66.58 ± 30.95
α (1/h)	10.87 ± 7.06	6.63 ± 3.28	6.88 ± 4.65	1.43 ± 0.68*
B (ng/ml)	43.58 ± 6.45	68.56 ± 17.89*	26.02 ± 3.61	11.53 ± 6.64*
β (1/h)	0.67 ± 0.21	0.35 ± 0.15*	0.70 ± 0.30	0.24 ± 0.21
V_c (l)[b]	0.34 ± 0.10	0.45 ± 0.26	–	–
$t_{1/2\alpha}$ (h)	0.09 ± 0.05	0.13 ± 0.08	0.18 ± 0.18	0.50 ± 0.38
$t_{1/2\beta}$ (h)	1.09 ± 0.27	2.32 ± 1.14	1.27 ± 0.86	4.78 ± 3.41
K_{21} (1/h)	3.29 ± 1.12	2.16 ± 2.41	3.31 ± 1.90	0.24 ± 0.20*
K_{10} (1/h)	1.99 ± 0.62	1.23 ± 0.38	1.37 ± 0.40	0.36 ± 0.16*
K_{12} (1/h)	6.26 ± 5.46	3.66 ± 1.77	2.90 ± 3.11	0.61 ± 0.27
CL (l/h)	0.63 ± 0.12	0.63 ± 0.16	1.39 ± 0.41	0.74 ± 0.28*
Ka (IV) (1/h)	–	–	18.83 ± 8.59	19.78 ± 5.78
$t_{1/2ka}$ (IV) (h)	–	–	0.03 ± 0.02	0.04 ± 0.01
Ka (IN) (1/h)[c]	16.18 ± 8.59	13.53 ± 8.59	2.67 ± 0.48	2.06 ± 1.08
$t_{1/2ka}$ (IN) (h)[c]	0.06 ± 0.03	0.07 ± 0.04	0.27 ± 0.05	0.44 ± 0.26
Ka (IG) (1/h)[d]	–	2.47 ± 1.52	–	1.31 ± 1.07
$t_{1/2ka}$ (IG) (h)[d]	–	0.38 ± 0.23	–	0.46 ± 0.15

[a] Parameters from A to CL were calculated from IV administration.
[b] V_c (CSF) calculated from direct lateral compartment injection was 135 ± 22 μl.
[c] Calculated from IN administration.
[d] Calculated from IG administration.
* $p < 0.05$ vs. 167 μg/ml group.

Table 3
AUC values for Huperzine A following intranasal, intravenous and intragastric administration

Dose (μg/kg)	AUC_{plasma} (ng h/ml)	AUC_{CSF} (ng h/ml)	Ratio of AUC_{plasm} (IN/IV or IG/IV)	Ratio of AUC_{CSF} (IN/IV or IG/IV)	Ratio of AUC (CSF/plasma)	Dose percentage in CSF (%)[a]
167 (IV)	112.8	37.9	–	–	0.336	0.087
500 (IV)	219.7	129.6	–	–	0.590	0.099
167 (IN)	101.9	48.4	0.903	1.277	0.475	0.111
500 (IN)	200.6	89.9	0.913	0.694	0.448	0.069
500 (IG)	217.3	67.6	0.989	0.521	0.311	0.052

[a] Calculated by comparing the AUC_{CSF} of various administrations with the AUC_{CSF} (4373.3 ng h/ml) following direct lateral compartment injection (dose 5 μg).

and sialorrhea. These rapid onsets on the body tissues showed the Huperzine A had good penetration ability among them. Following direct lateral compartment injection (5 μg), the V_c was 135 μl, the CL was 1.2 ml/h, the AUC_{CSF} was 4373.3 ng h/ml.

4. Discussion

Since 1960s, the researchers were trying to explore the factors that decided the capabilities of compounds passing across the BBB into the CNS from the blood (Brodie et al., 1960; McMartin et al., 1987; Sakane et al., 1991, 1994, 1995, 1997). They found the factors such as the lipophilicity, dissociation constant and molecular weight played an important role in drug transportation abilities. With such physicochemical properties: molecular weight of 242.32, pK_a of 6.18, apparent solubility of 0.93–1.43 mg/ml (in pH 7.0–7.7 PBS, 25 °C) and log DC of 0.78–1.15 (between octanol and PBS pH 7.0–7.7), the Huperzine A was supposed to be capable of going across the BBB readily under passive diffusion mechanism which could be proved by the rapid onset in CSF shown in Figs. 1 and 2 in which T_{max} (CSF) following intravenous administration was <3 min in this research. And the observed cholinergic side-effects such as muscle trepidation, sialorrhea, miosis and defecation etc all over the body appeared within several minutes after administration showed that the drug had very powerful permeation ability among body tissues.

Various drugs have been studied following intranasal administration compared with intravenous administration to convince the existence of the direct rout from nasal cavity to the CNS. Two transport pathways via the nasal olfactory epithelium into the CSF (subarachnoid space and axoplasmic flow of olfactory nerve) and three transport processes (passive diffusion, carrier mediated and bulk flow) have been proposed and summarized by previous investigators. Some of the studies showed higher drug concentrations following intranasal administration than intravenous administration or even incredible higher drug concentrations in CSF than in the plasma following intranasal administration. In this study, the above phenomena were not remarkably observed, while drug concentrations in CSF following intranasal administration were slightly higher than following intravenous administration from 30 min to 6 h in 167 μg/kg dose group. And this superiority finally contributed to the ratio 1.28 of AUC_{CSF} (IN/IV). Results are shown in Table 3. The absorption rate of intranasal administration into blood and CSF were both obviously quit swift which led to both similar plasma concentrations at 15 min and CSF concentrations at 30 min with intravenous administration, but compared with the CSF concnetrations of intravenous administration the nasal rout was still lower at the first few points, which meant if the direct transport route into the CNS from the nasal cavity exist, it needs time to work. In another word, although intranasal administration had direct rout to deliver drug into CNS, it is not equal to injecting drugs into the brain directly.

The observed AUC_{CSF} ratios in this study provide supportive evidence that a certain portion of drugs were carried through the olfactory region directly into the CSF. But confusing results also appeared that when the dose was three times changed from 167 to 500 μg/kg the AUC_{CSF} ratios (IV/IN) declined from 1.28 to 0.69. Furthermore, significances of some parameters in CSF between the two doses and huge differences on the ratio of AUC (CSF/plasma) were found. For intranasal administration, the ratio of AUC (CSF/plasma) were 0.475–0.448, while following intravenous administration they were 0.336–0.590, nearly twice lifted. And dramatic changes in the CSF disposal of drugs following intravenous administration (500 μg/kg) were: for example, in plasma the $t_{1/2\alpha}$ and $t_{1/2\beta}$ of the two doses following intravenous administration were 0.09–0.13 and 1.09–2.32, but in CSF they were 0.18–0.50 and 1.27–4.78, and the CL (l/h) in plasma of both doses was the same (0.63), but in CSF it shrink nearly twice (1.39–0.74). The above facts meant that the disposal ability of the CNS to the drug was challenged, the elimination rate of Huperzine A in the CSF decreased and the $t_{1/2}$ was prolonged as the dose increasing. So the drug concentrations in the CSF following the intravenous administration in dose (500 μg/kg) was excessively high and could maintain a quite long time when the elimination ability in CSF was saturated, this caused the surprising and abnormally big AUC_{CSF}. In other word, this dose (500 μg/kg) was not chosen cautiously without fully consideration of the LD50 or toxicity on rats in such a high dose. So, before we started any pharmacokinetic and neuropharmacokinetic researches, it was very important that the proper dose was carefully selected and paid attentions to in consideration of the animal metabolism ability.

For intranasal administration the differences between the two doses were merely the volumes to be administrated: 25 μl for 167 μg/ml and 75 μl for 500 μg/kg. Supposing the area in the nasal cavity of rat to deliver drugs to the CNS was determinate, when the area was saturated the superfluous drug could only be absorbed into the blood by respiratory membrane. In addition there are many differences in morphologic structure between human and rats. In future applications the distinctness between spray in human and injection into rat's nostril will obvi-

ously exist. So, further researches in bigger animals and spraying administration are exactly needed. Although Huperzine A displayed very good permeation through the BBB and among body tissues, some studies showed drug in CSF could only distributed on superficial brain while not in deep brain cortex. To elucidate that, researches on distribution were needed for more supports in later study.

On the whole, the intranasal administration was an attractive non-invasive alternative for the Huperzine A which provided similar plasma concentrations after 15 min and higher CSF concentrations after 30 min than intravenous administration.

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