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ScienceDirect
International Journal of Pharmaceutics 328 (2007) 161–167
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Delivery of ^{125}I-cobrotoxin after intranasal administration to the brain: A microdialysis study in freely moving rats

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Received 23 February 2006; received in revised form 3 July 2006; accepted 8 August 2006
Available online 18 August 2006

Abstract

In order to determine the contribution of intranasal (i.n.) administration to the uptake of large molecular weight (MW) substances into central nervous system (CNS), concentration in brain of the centrally acting polypeptide cobrotoxin (NT-I) versus time profiles were studied using dual-probe microdialysis in awake free-moving rats. NT-I, radiolabeled with sodium ^{125}I-Iodide (^{125}I-NT-I), was administered at the dose of 105 μg/kg intravenously and intranasally in the same set of rat ($n = 15$). The ^{125}I-NT-Inasal preparations were formulated with borneol/menthol eutectic mixture (+BMEM) as an absorption enhancer and without (−BMEM). After application, the dialysates sampled simultaneously from olfactory bulb and cerebellar nuclei were measured in a gamma-counter for radioactivity. The real concentrations of NT-I were recalculated by *in vivo* recoveries of microdialysis probes. The results showed that the area under the curve (AUC) value in cerebellar nuclei (2283.51 ± 34.54 min ng/ml) following i.n. administration (+BMEM) was significantly larger than those ($AUC_{olfactory}$ = 1141.92 ± 26.42 min ng/ml; $AUC_{cerebellar}$ = 1364.62 ± 19.35 min ng/ml) after intravenous (i.v.) bolus, respectively. A prolonged time values to peak concentrations after i.n. application (+BMEM) were observed compared with those following i.v. administration. Also, following i.n. application (+BMEM) the measured time value to peak concentration in cerebellar nuclei (85 min) was statistically longer than that in olfactory bulb (75 min), which could be plausibly an indication for NT-I delivery into brain via nose–brain pathway in the presence of absorption enhancer. i.n. administration (−BMEM) had little or no ability of NT-I delivering into brain. In conclusion, i.n. administration (+BMEM) significantly enhanced brain transport of NT-I with uneven distribution in discrete regions of brain compared with i.v. administration. Additionally, multi-probe microdialysis technique should be considerably valuable in brain delivery studies.

Keywords: Cobrotoxin; Microdialysis; Nose–brain pathway; Intranasal administration; Rats

1. Introduction

NT-I, a short-chain neurotoxin, is the main neurotoxic protein identified from the venom of the monocled cobra (Tsetlin, 1999; Lu et al., 2002). NT-I is believed to be a promising candidate for analgesic therapeutics and the biological treatment of drug addiction (Xiong and Wang, 1992). Based on Xiong and Wang (1992) and Yang (1999) experimental and clinical study had revealed that injections of cobrotoxin brought about no additive and produced definite relief of pain, which is more slowly but proved and more lasting compared with that pronounced by morphine. Little is elucidated to present concerning the analgesic mechanism of NT-I, but cerebrum was proposed to be the target site of the analgesia action (Phui Yee et al., 2004). However, a significant challenge to its clinical application is the limitation associated with delivering these polypeptides to the central nervous system (CNS).

Intranasal administration is often considered as a safe and acceptable route of drug delivery of brain targeting, circumventing the blood–brain barrier (BBB), especially for the substances with biological effects on the CNS and limited BBB permeability (Illum, 2000, 2002). Undoubtedly, i.n. administration is of greater significant for peptide and protein drugs than those of small molecule for their limited transportation into brain via BBB from systemic circulation. The previously investigated large molecule models in experimental animals extensively include nerve growth factor (NGF), insulin, desmopressin, cholecystokinin and insulin-like growth factor-1 (Harris et al., 1986; Frey et al., 1997; Chen et al., 1998; Liu et al., 2001a,b; Giacobini et al., 2004). Evidently, many brain target-

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0378-5173/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2006.08.011

ing researches of large MW molecules have demonstrated the potential of nose–brain pathway (Frey et al., 1997; Chen et al., 1998; Giacobini et al., 2004; Yu et al., 2004). Nevertheless, several other studies (Bagger and Bechgaard, 2004; in 't Veen et al., 2005) drew the opposite conclusion that additional direct delivery from nasal mucosae into CNS was limited. Illum (2003) demonstrated that the two most important factors contributing to limited nasal absorption of large molecule polar drugs are the low membrane permeability and the rapid mucociliary clearance of drugs intranasally administered. As for the former, novel drug formulation system, such as drug-loaded microspheres (Li et al., 2005), microemulsion (Zhang et al., 2004), and absorption promoting agents, like chitosan (Yu et al., 2004), poly-L-arginine (Ohtake et al., 2002), bornoel, are all applicable to improve drug nasal transmucosal absorption with good complication. Considering the rapid mucociliary clearance, a design using an awake animal model in the study is also suggested to be employed due to an effect of the anaesthetic agents to a variable degree on the mucociliary clearance (Mayor and Illum, 1997), in order to make the experimental data on nose–brain delivery more conformable to physiological condition.

Microdialysis sampling technique is believed to be a unique tool to assay unbound drug disposition and metabolism in brain that is gaining popularity in pharmacokinetic studies (Elmquist and Sawchuk, 1997; De Lange et al., 2000; Tsai, 2003). Compared with conventional sampling methods, including ultrafiltration, equilibrium dialysis and brain tissue hemogenization (Wang et al., 1998; Chow et al., 2001), brain microdialysis overcomes the disadvantages induced by these techniques of, either perturbation of physiological function or analysis based on composite data.

As far as we are aware the present investigation was first carried out using microdialysis in a conscious rat model to evaluate the potential of brain delivery of nasally administered ^{125}I-cobrotoxin (^{125}I-NT-I) in combination with eutectic mixture of borneol/menthol (1:3, w/w) as an absorption enhancer in order to facilitate absorption of ^{125}I-NT-I from nasal cavity directly into the central nervous system. Several investigations focusing on the physicochemical and pharmacokinetic studies of eutectic mixture binary melt systems had also been undertaken (Stott et al., 1998; Kang et al., 2000; Chen et al., 2004; Yong et al., 2004a,b). In our other studies it had been observed that the BMEM, administered nasally with drug together in rats, produced a good compliance and pharmacokinetically a relatively higher absorption enhancement than single borneol or menthol for polypeptide molecule delivering from nares to brain, which will be discussed in detail in our other paper. In the present work borneol was used at a composition of approximately 0.12% in the ^{125}I-NT-I solution nasally administered with BMEM.

For comparison ^{125}I-NT-I was also administered intravenously. As a control experimental animals were dosed nasally with ^{125}I-NT-I without absorption enhancer. No group was given anaesthetic before administration and during the course of study. In this study, the concentration–time profiles of ^{125}I-NT-I in olfactory bulb and cerebellar nuclei were determined by radioactivity measurement.

2. Experimental

2.1. Chemicals

NT-I (molecular weight: 6952.19 Da) with purity of approximate 99.8% was kindly provided by Department of Animal Toxicology, Kunming Institute of Zoology, Chinese Academy of Sciences (Yunnan, China). Borneol (purum, ≥98.0%) and menthol (purum, ≥98.5%) were purchased from Sigma–Aldrich China Inc. (Shanghai, China). Sterile sodium chloride solution 0.9% and liquid paraffin reagents (Shanghai Chemical Reagents Co., China) were used as dissolvent for the intranasal and intravenous formulation preparation. All other chemicals were of analytical grade and commercially available.

2.2. Animals

Fifteen male adult Sprague–Dawley rats weighing approximately 350 g were obtained from the Laboratory Animal Center of Zhejiang Academy of Medical Sciences (Zhejiang, China). Animals were acclimatized for at least 5 days with alternating dark/light cycle of each 12 h in a climate controlled room with temperature maintained at 22 ± 1 °C and a relative humidity of 60 ± 10%. Water and standard laboratory food were available *ad libitum*. All experiments were performed according to the guidelines for the care and use of animals as established by Zhejiang University.

2.3. Methods

2.3.1. Iodination of ^{125}I-NT-I

NT-I was radiolabeled with sodium ^{125}I-iodide (Gaotong Isotope Co., China) using Iodogen method. Briefly, NT-I (110 μg; 2.5 mg/ml) solution was mixed with phosphate buffer (pH 7.4; 300 μl) and sodium ^{125}I (1.0 mCi; 10 μl) in an Eppendorf tube. Reaction was initiated by addition of one Iodo-bead®. Then, the reaction mixture was loaded onto a Sephadex G215 (1 cm × 25 cm) packed from Pharmacia and purified to obtain the mono-iodinated product of 62.54 Ci/mmol (81 μl; 100 μg NT-I) using a linear gradient with 50–100% acetonitrile against 0.1% trifluoroacetic acid (TFA). The radioactivity was assessed in a SN-682 gamma-counter (Rihuan instrument factory of Shanghai Atomic Nuclide Institute, China). The radiochemical purity was 99.1%. The assessment efficiency coefficient of radioactivity is 0.44.

2.3.2. Preparation of borneol/menthol eutectic mixture solution

Borneol and menthol in weight radio of 1:3 were thoroughly and continuously grinded using pestle and mortar at room temperature until a clear homogenous eutectic mixture in a liquid state was obtained. The hydrophobic eutectic mixture was added in liquid paraffin, in which the borneol/menthol eutectic mixture solution was prepared containing approximately 1.2% (w/v) of borneol as a concentration marker. This solution was diluted in ^{125}I-NT-I solution to a final borneol concentration of 0.12% (w/v) just prior to i.n. administration.

2.3.3. Surgical procedures

Sprague–Dawley rats were anesthetized with ketamine/xylazine (90 + 10 mg/kg) by intraperitoneal injection and mounted on a stereotaxic frame (Bioanalytical Systems, West Lafayette, IN, USA). The surgical procedure was described elsewhere (Bergquist et al., 1996). Briefly, two 0.5 mm holes in a rat were drilled in the skull separately (right olfactory bulb: −1.0 mm lateral to the midsagittal suture and 6.0 mm anterior to bregma; cerebellar nuclei: −2.3 mm lateral to the midsagittal suture and 11.6 mm posterior to bregma). Two chronic brain microdialysis guide cannulae (MD-2251, BAS, West Lafayette, IN, USA) with styles in place were inserted into the brain aimed to the right olfactory bulb and cerebellar nuclei, respectively, identically to a depth of 4.6 mm ventrally from the dura according to the Rat Brain Atlas of Paxinos and Watson (Paxinos and Watson, 1997). The guide cannulae were fastened to the cranium with skull screws and dental acrylic cement. After the surgery the animals were allowed to recover for 6 days in single cages under standard conditions (12 h light/dark cycle, a controlled ambient temperature of 22 ± 2 °C and a relative humidity of 60 ± 10%), with free access to food and water until 24 h before administration, at which time only food was withdrawn.

2.3.4. Recovery determination

The *in vitro* and *in vivo* retrodialysis recoveries of the drug ^{125}I-NT-I were both determined as described below prior to probe implantation. The *in vivo* retrodialysis recoveries by loss of ^{125}I-NT-I were used for further recalculations of true extracellular drug concentrations in sampling sites, olfactory bulb and cerebellar nuclei, respectively.

Both *in vitro* recoveries obtained by gain and loss (retrodialysis) before the rat experiments of three probes of the same type for use in brain (MD-2200, BAS, West Lafayette, IN, USA) with characteristics of 0.5 mm O.D., 2 mm membrane length and nominal 38 kDa MW cut-off with four concentration levels (1, 5, 10 and 15 ng/ml), were investigated at 2.0 μl/min flow rate. Thus, the *in vitro* recoveries (gain and loss) for ^{125}I-NT-I were 25.4% and 28.7%, respectively, between which no significant difference ($P > 0.05$) was found. No difference between the three individual probes was shown ($P > 0.05$). The *in vitro* recoveries (gain and loss) were used only to evaluate the functional properties of each probe.

The *in vivo* recovery by loss of ^{125}I-NT-I for each probe at both olfactory bulb and cerebellar nuclei was calculated from the following equation:

$$\text{recovery } (^{125}\text{I-NT-I}) = \frac{C_{\text{NT dialysate}} - C_{\text{NT perfusate}}}{C_{\text{NT BIF}} - C_{\text{NT perfusate}}},$$

where C_{NT} is the ^{125}I-NT-I concentration, given for dialysate, perfusate and BIF (brain interstitial fluid), respectively. During the retrodialysis period, the concentration of ^{125}I-NT-I in BIF was assumed to be zero due to its relative high diffusion coefficient in brain tissues. Three of the samples (20 μl per sample) from each period were collected and analyzed over 10 min intervals (first two and last samples discarded). The *in vivo* recovery ($n = 12$) of ^{125}I-NT-I (loss) was also conducted on the same type probes as those in *in vitro* recovery experiments with four concentration levels (1, 5, 10 and 15 ng/ml). All statistical analyses (ANOVA) were performed by Origin 6.0 (Microcal Software, Inc., MA, USA).

2.3.5. Microdialysis experiments

Fifteen rats were randomly divided into three groups ($n = 5$ per group). One group of animals were administered intranasally at a dose of 85 μl/kg ^{125}I-NT-I (105 μg; 944 μCi/kg rat) with proportional BMEM solution (final borneol concentration of 0.12% (w/v)). The control group received i.n. administration at the same dose of ^{125}I-NT-I in the absence of enhancers. In the i.v. group, equal dose of ^{125}I-NT-I was injected intravenously via tail vein. In a supine-70 position was the rat under controlled using a home-made patent device when all nasal formulations were dosed. Nasal preparations were administered with a soft PE-10 tubing (Becton, Dickinson and Company, USA) fitted to a 50 μl Hamilton microsyringe, inserting 15 mm into the cavity in the right nostril (Bagger and Bechgaard, 2004). All microdialysis experiments were performed in awake freely moving animals kept in an awake animal caging system (Stand-Alone Raturn and Rodent Bowl kit, BAS, West Lafayette, IN, USA), with no anaesthesia used throughout the experiment.

On the day of the experiment two brain microdialysis probes (MD-2200) were inserted into the two guide cannulae, respectively, 30 min before sampling was begun. The inlets of the probes were both attached to a BAS syringe driver (MD-1001, USA) connected with a controller (240V/50 Hz MD-1000K, BAS, West Lafayette, IN, USA), filled with a modified Ringer's solution as perfusion fluid (145 mM NaCl, 3.7 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 10 mM $NaHCO_3$, 0.1 mM ascorbic acid, adjusted to pH 7.4). The brain microdialysis probes were simultaneously perfused at a 2 μl/min flow rate for 30 min (delay time) and all microdialysates collected were discarded in order to eliminate the void volume in tubing and stabilize solutes levels around the dialysis membrane before starting the collecting of samples. Posterior to this equilibrium period, brain microdialysate samples were collected using refrigerated fraction collector (MD-1201, BAS, West Lafayette, IN, USA) into 300 μl vials under a 10 min sampling regime throughout the experiments (sample volume = 20 μl per vial).

Intravenously or intranasally the experimental animals were dosed in the conscious condition. Simultaneously, brain microdialysate samples from the right olfactory bulb and cerebellar nuclei, respectively, were collected for an additional 300 min automatically.

After each experiment, the animals were anesthetized with excessive chloral hydrate and transcardially perfused with 4% paraformaldehyde in 0.1 M Sorenson'buffer as described previously (Williams et al., 1995). The brains were dissected out, immediately frozen and stored at −20 °C. Coronal 40 μm thick slices were later cut in a cryo-microtome and the unstained sections were observed under magnification to localize the probe tract. Only animals with probe located in the olfactory bulb and cerebellar nuclei were included in the present study—others were not assayed.

2.3.6. Data analysis

The area under the olfactory bulb or cerebellar nuclei extracellular unbound concentration versus time curve values from 0 to 5 h were determined using the trapezoidal rule. Mean AUC values and $AUC_{i.n.}/AUC_{i.v.}$ ratios were calculated for both two routes of administration and two probed brain regions to determine the direct brain delivery of NT-I from nose. The values of K_{ab} were calculated by method of residuals. Statistical analyses (ANOVA) of mean results across multiple treatment groups were performed by Origin 6.0 (Microcal Software, Inc., MA, USA), followed by paired Student's *t*-test. A *P*-value below 0.05 was taken to indicate significant difference between data means. All values were presented as mean ± S.D.

3. Results

Average recovery by loss (retrodialysis) *in vivo* ($n = 12$) of three brain probes (MD-2200) with four concentration levels of ^{125}I-NT-I used in right olfactory bulb of rat was 14.1% (S.D. ± 1.2%), whereas average recovery by loss in cerebellar nuclei was 14.8% (S.D. ± 1.7%) ($P > 0.05$). This was used to correct the actual concentration of NT-I in dialysates sampled from right olfactory bulb and cerebellar nuclei in rats.

Concentration of polypeptide NT-I versus time profiles in the right olfactory bulb and cerebellar nuclei, respectively, following i.n. (+BMEM) and i.v. administration to conscious rat were presented in Fig. 1, in which almost identical concentration–time profiles were found after i.v. dosing compared with that after i.n. administration (+BMEM). The comparable delivery of NT-I into the right olfactory bulb and cerebellar nuclei, respectively, was also obtained after both routes of administration (i.v. and i.n. with BMEM), whereas little distribution of NT-I from nose into probed brain tissues following nasal application without enhancer was observed (data not shown). This suggests evidently that there is no uptake of polypeptide NT-I from nose to brain administered intranasally without absorption enhancer.

From Fig. 1, it was found that intravenous administration resulted in the maximum measured extracellular concentrations of 11.49 ± 2.5 and 11.74 ± 0.8 ng/ml in the right olfactory bulb and cerebellar nuclei, respectively, which were both much close to that in the cerebellar nuclei after nasal (+BMEM) administration ($P > 0.05$). Compared with a continuously gentle declines following the peak concentrations of intravenous ^{125}I-NT-I, there were plateaus from 95 min to the last time point in the right olfactory bulb and between 105 and 215 min in the cerebellar nuclei followed by a quite slow decline after nasal (+BMEM) application. Obviously, cerebellar nuclei levels of ^{125}I-NT-I after 35 min post-i.n. administration (+BMEM) were always higher than right olfactory bulb concentrations till the final microdialysis point, especially the maximum measured concentration with significant difference ($P < 0.05$). Following an i.v. bolus, the measured time to maximum concentrations was 25 min identically in both different probed brain regions. In contrast, nasal administration produced peak concentration at 75 min post-application in the right olfactory bulb, which was interestingly shorter than that (85 min) in the cerebellar nuclei. Generally, the times to peak concentrations in awake and intranasally administered rats were significantly longer than those achieved in awake and intranvenously administered rats. These results demonstrate that NT-I intranasally administered may be delivered directly into brain via the nose–brain route.

Accordingly, the apparent availabilities ($AUC_{i.n.}/AUC_{i.v.}$) were 119% and 167% in the right olfactory bulb and cerebellar nuclei, respectively. Other pharmacokinetic parameters are presented in Table 1. There was statistical difference between the area under the curve (AUC) of NT-I i.n. administration (+BMEM) and i.v. administration in the cerebellar nuclei. Also, the AUC of cerebellar nuclei curve was significant different with that of olfactory bulb curve after nasal application.

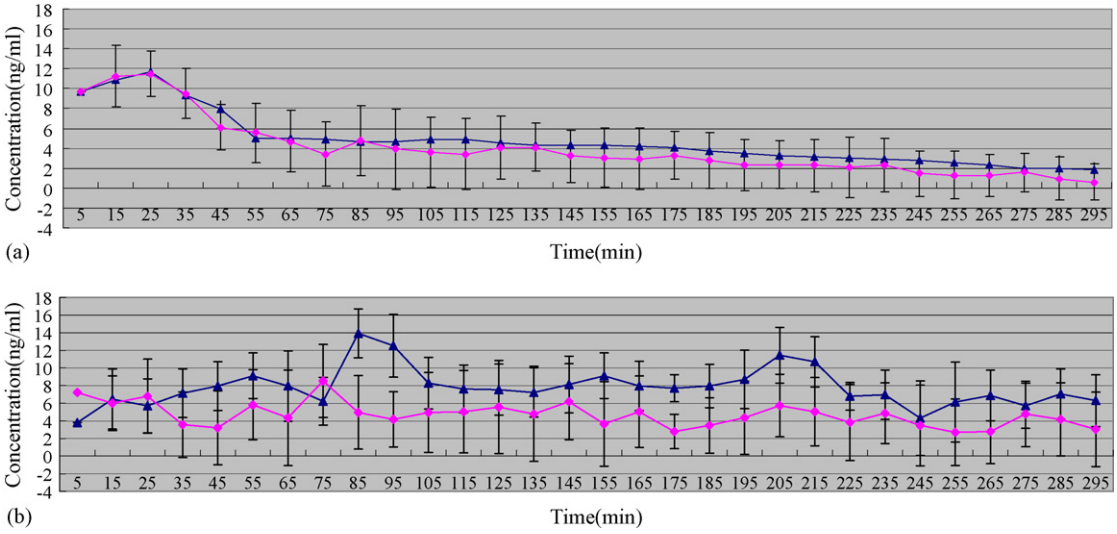

Fig. 1. NT-I concentration–time profiles in the right olfactory bulb (♦) and cerebellar nuclei (▲), respectively, after i.n. and i.v. administration to awake rat. Results are presented as mean ± S.D: (a) i.v., 105 μg/kg ($n = 5$); (b) i.n., 105 μg/kg (+BMEM) ($n = 5$). Bars indicate standard deviations.

Table 1
Pharmacokinetic analysis of 105 μg/kg NT-I administered intravenously (i.v.) and intranasally (i.n.) with BMEM in conscious rat brain

Parameters	i.n. (+BMEM)	i.v.	Ratio (i.n./i.v.)
C_{max} (cerebellum, ng/ml)	13.93 ± 3.55	11.74 ± 0.8	
C_{max} (olfactory, ng/ml)	8.53 ± 4.17	11.49 ± 2.5	
T_{max} (cerebellum, min)	85	25	
T_{max} (olfactory, min)	75	25	
K_{ab} (cerebellum, h^{-1})	0.016	0.019	
K_{ab} (olfactory, h^{-1})	0.029	0.024	
$AUC_{0-5\,h}$ (cerebellum, min ng/ml)	2283.51 ± 34.54	1364.62 ± 19.35*	1.67
$AUC_{0-5\,h}$ (olfactory, min ng/ml)	1358.58 ± 32.56*	1141.92 ± 26.42	1.19
$AUC_{0-\alpha}$ (cerebellum, min ng/ml)	6412.12 ± 73.11	1719.5 ± 26.21	
$AUC_{0-\alpha}$ (olfactory, min ng/ml)	4351.31 ± 46.25	1318.7 ± 13.89	

Data are expressed as mean ± S.D. ($n = 10$).
* Significant difference ($P < 0.05$) vs. $AUC_{cerebellar}$ of NT-I i.n. administration (+BMEM).

4. Discussion

Centrally active protein and peptide agents with poor bioavailability and severe limitation of BBB penetration have been increasingly developed, which makes the administration mode more challenging. Recent developments in nasal drug delivery have gradually revealed that nasal administration is a promising alternative to conventional invasion administration routes (Shi et al., 2005), especially for large MW drugs (Thorne and Frey, 2001; Illum, 2003). The present results also suggest that nasal administration in presence of absorption enhancer did exert some distribution advantage for NT-I into the brain over systemic dosing, while NT-I intranasally administered in absence of absorption enhancer had little or no potential of delivering into the CNS.

Previous studies by Yang (1999) and Lu et al. (2002) demonstrated that NT-I is basic (p$I > 7$), small MW polypeptide at physiological pH, comprised of 62 amino acid residues including tyrosine which is the most suitable isotope labeling site for sodium ^{125}I-iodide. ^{125}I-NT-I represented stable radioactivity during the experiment period. The brain distribution results of the present study showed that after i.v. bolus injection the time to peak levels in two probed brain regions were both just approximately 25 min subsequently followed by a relative slow concentration decline due to clearance of NT-I in the brain. Also, the extent of cerebellar nuclei distribution was significantly similar to that of olfactory bulb after a dose of NT-I injection.

These results indicated that NT-I penetrated into the brain via the BBB to an appreciable extent from systemic circulation. Banks et al. (1997) and Pan et al. (1998) had found that polypeptide neurotrophins and insulin have the ability of permeation across the blood–brain barrier by saturable transport systems. Simple diffusion and saturable transport systems were considered to be two mechanism for central nervous system penetration of all polypeptides (Kastin et al., 1999), and the latter is presumed to contribute to the NT-I delivery from the periphery into the brain via BBB.

According to the studies by in 't Veen et al. (2005), a prolonged period of absorption ($T_{max} = 90$ min) following nasal administration of FD3, a high MW compound, was found. Similarly, in the present study the peak measured concentrations in two different brain tissues were attained at 75 and 85 min, respectively, after polypeptide NT-I i.n. application, evidently suggesting a longer absorption phase compared with that following an i.v. injection. However, the studies by Frey et al. (1995, 1997) demonstrated that nerve growth factor labelled with ^{125}I achieved maximum concentration in the olfactory bulb 20 min following nasal application and to a lesser extent in the other parts of the brain. These observations are not completely consistent with our findings suggesting a longer absorption phase. This is plausibly attribute to the combining application of absorption enhancer BMEM which may increase the residence time of the formulation in the nasal cavity and transiently open the tight junctions between the cells, though its real mechanism of absorption enhancing are still unclear. Accordingly, three nose–brain pathways are proposed to be modes for a drug to reach the CNS from the nasal cavity, which are transcellular pathway, paracellular pathway and intracellular axonal transport (Illum, 2003). Considering the time to maximum level, $AUC_{i.n.}/AUC_{i.v.}$ ratios and its hydrophilia comprehensively, it is more probable that NT-I after nasal administration in combination of absorption promoting agent was delivered into brain paracellularly.

In the present study, a hysteresis was interestedly observed between the time to peak concentration values in cerebellar nuclei and in olfactory bulb following i.n. administration, whereas there is no significant difference among those after a i.v bolus. This could be an indication that the drug was transported across the nasal membrane into the olfactory lobe and then distributed into other brain tissues, i.e., direct nose-brain transport exited in nasal substance NT-I.

Based on the review by Illum (2002), three major influence factors associated with the capability of substances delivery across the nose mucosa into brain were low membrane permeability, the mucociliary clearance mechanism and a possible enzymatic degradation of the drug in the nasal cavity, respectively. The study by Mayor and Illum (1997) suggested that the use of anaesthetics was proposed to a variable extent to attenuate the ability of the nasal mucociliary clearance. As a result, a prolonged residence time of nasal formulation in the nasal cavity was obtained. In this paper, a free-moving rat model with no anaesthesia introduced was utilized throughout the experiment in order to deplete the influence of anaesthesia on absorption

via nasal mucous membrane, with access to physiological condition. Borneol and menthol are used individually as mucosa absorption enhancers extensively (Kang et al., 2000; Yong et al., 2004a,b). In our other study, for BMEM an effective ability of mucosa absorption enhancing with good compliance was observed. According to Illum (2003), the mechanisms of the absorption promoting systems generally include permeability enhancement of the nasal epithelial cell layer by modifying the phospholipid bilayer, tight junctions opening and enzymatic inhibition. Therefore, BMEM was applied to the present study to improve the NT-I nasal absorption. Our experiment results also determined that absorption enhancer was a key case in delivery to brain following i.n. application of NT-I.

Microdialysis sampling technique, causing minimal perturbation to physiological processes, has been to date extensively used in and greatly contributory to neurosciences, pharmacodynamics and drug disposition and metabolism researches (Davies, 1999), in which brain microdialysis study is especially of significance in that traditional brain research methods exhibit evidently more disadvantages. Several exclusive characteristics of microdialysis make it quite suitable for drug delivery research, specifically in the brain. In contrast to single brain probe sampling, application in brain delivery study of dual- and triple-probe microdialysis simultaneously in the same experimental animal brain is seldom reported. Although two investigations on drug diffusion in rat brain after intracerebrally infused (Hoistad et al., 2000, 2002) using dual-probe microdialysis technique were conducted, there is no multi-probe microdialysis employed to present in drug brain targeting studies. In the present study, dual-probe microdialysis was employed in intranasal drug delivery for brain targeting study. The results indicated that using this technique a high time and spatial resolution of the distribution in the discrete brain areas of brain targeting drug following i.n. administration was exhibited.

In summary, it was proved that nasal administration in combination with BMEM significantly enhanced brain delivery of polypeptide NT-I with uneven distribution in different brain regions compared with intravenous administration. It is noteworthy that nasal administration in absence of absorption enhancer contrarily had little or no ability of NT-I delivering into brain. Also, a hysteresis of the time to maximum concentration occurred clearly in the cerebellar nuclei over that in the olfactory bulb. Thus, we could concluded prudently that an additional pathway from nose to CNS for NT-I in the presence of absorption enhancer exists. Additionally, multi-probe microdialysis technique should be considerably valuable in drug delivery for brain targeting studies.

Acknowledgements

We are grateful to Prof. Jianhua Zhu of Department of Radiopharmacy, School of Pharmacy of Fudan University for technical assistance in Iodination of ^{125}I-NT-I. Our thanks also to Dr. Fuming Xie from Bioanalytical Systems, Inc., USA for the scrupulous instruction on microdialysis technique. This work was financially supported by Project No. 30371781 of the National Natural Science Foundation of China.

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