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A novel method to calculate the extent and amount of drug transported into CSF after intranasal administration☆

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Abstract

The aim of this paper is to establish a novel method to calculate the extent and amount of drug transported to brain after administration. The cerebrospinal fluid (CSF) was chosen as the target region. The intranasal administration of meptazinol hydrochloride (MEP) was chosen as the model administration and intravenous administration was selected as reference. According to formula transform, the extent was measured by the equation of $(X_A)_{CSF,\infty}/X_0 = Cl_{CSF}\ (AUC_{0\rightarrow\infty})_{CSF}/X_0$ and the drug amount was calculated by multiplying the dose with the extent. The drug clearance in CSF (Cl_{CSF}) was calculated by a method, in which a certain volume of MEP solution was injected directly into rat cistern magna and then clearance was assessed as the reciprocal of the zeroth moment of a CSF level-time curve normalized for dose. In order to testify the accurateness of the method, ^{14}C-sucrose was chosen as reference because of its impermeable characteristic across blood-brain barrier (BBB). It was found out that the MEP concentrations in plasma and CSF after intranasal administration did not show significant difference with those after intravenous administration. However, the extent and amount of MEP transported to CSF was significantly lower compared with those to plasma after these two administrations. In conclusion, the method can be applied to measure the extent and amount of drug transported to CSF, which would be useful to evaluate brain-targeting drug delivery.

Keywords: Brain-targeting; Extent; Intranasal administration; Clearance; Cerebrospinal fluid; Meptazinol hydrochloride

1. Introduction

Blood-brain barrier (BBB) is essential in maintaining the stable internal environment, which permits the entry of necessary nutrients via specific channels and restricts the free diffusion of molecules across BBB into central nervous system (CNS). Meanwhile, the property of BBB limits transport of the therapeutic agents into brain to treat certain diseases such as Parkinson disease, brain tumor and Alzheimer disease, etc.

In the past two decades, different technologies have appeared to bypass BBB to deliver drugs into brain such as nanoparticle drug delivery system (Kreuter

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et al., 1995), vector-mediated drug delivery (Pardridge, 1999) and intranasal administration (Illum, 2000), etc. Since the report of Erlich et al. (1986) that there were no significant barrier between the nasal mucosa and brain at the rabbit cribriform region, and that the CSF reached the submucosal region rapidly via open pathways in this model, there has been more and more substances showing the direct transport to brain via nasal mucosa including local anesthetics (Chou and Donovan, 1998), 5-fluorouracil (Sakane et al., 1999), cocaine and benzoylecgonine (Chow et al., 2001) and insulin-like growth factor-I (Liu et al., 2001). Additionally, there are so far several commercial products in the market (Illum, 2003) including anti-migraine drugs like sumatriptan and a range of peptides such as calcitonin, desmopressin and buserelin, etc.

In order to confirm the existence of olfactory pathway, a lot of methods were adopted including the apparent brain uptake clearances (Sakane et al., 1999), plasma-to-brain permeability × area product (Wang et al., 1998) and drug targeting index (Wang et al., 2003), etc. Although these methods have been testified their efficacy, they are all based on the concentration–time profiles. And there is no such report to assess the extent and provide information of drug amount transported to CNS.

Therefore, the aim of our research is to establish a simple method to assess the extent and amount of drug transported into brain after intranasal administration. Since CSF flows over the surface of the brain and drugs can diffuse into the extracellular fluid of brain more easily from CSF than from the blood (Sakane et al., 1999), drug disposition in CSF can to some degree represent the pharmacokinetic behavior in brain. Meanwhile, there are a lot of target receptors on brain surface and their therapeutic effect is related to brain functions such as Parkinson's disease, treatment of Alzheimer's disease and the treatment of Pain (Illum, 2000). Thus, CSF was chosen as the target region in our experiment. Through formula transform, the extent transported to CSF was calculated according to the equation of $(X_A)_{CSF,\infty}/X_0 = Cl_{CSF}\,(AUC_{0\to\infty})_{CSF}/X_0$. The drug clearance was assessed by the statistical moment theory and validated by using ^{14}C-sucrose as the reference. The drug amount was measured by multiplying the dose with the extent. Meanwhile, the extent and amount of drug transported to plasma was also assessed. The intranasal administration of an analgesic, meptazinol hydrochloride (MEP), was selected as our model administration. MEP is a centrally-acting analgesic for the control of mild to severe pain in man. Its usual administration is oral administration. In addition, intravenous administration of MEP was also performed to measure the extent and drug amount as reference to confirm the existence of the olfactory pathway.

2. Materials and methods

2.1. Drugs and chemicals

Meptazinol hydrochloride (MEP) was supplied by the Department of Medicinal Chemistry, School of Pharmacy, Fudan University (Shanghai, China). MEP formulations for different administration were prepared by dissolving the drug into water and the concentration of intra-cistern magna, intranasal and intravenous formulation was 800 μg/ml, 80 mg/ml and 1.6 mg/ml, respectively. ^{14}C-sucrose (200 μCi/ml) was purchased from Amersham Biosciences Ltd., UK and diluted to 40 μCi/ml for experimental usage. Sodium pentobarbital (Shanghai Chemical Reagents Corp., China) was used as anesthetics for animal experiments. Millipore ultrapure deionized water (Millipore Simpli Lab, USA) was used for preparing MEP formulations and buffer of HPLC mobile phase. HPLC grade methanol was purchased from Shanghai Chemical Reagents Research Institute, China. All other chemicals were of commercially analytical grade.

2.2. Animal preparation

The animal experiment was carried out in compliance with the protocol of Animal Use and Care by Medical Center of Fudan University. Male Sprague–Dawley rats with an average weight of 280.5 g (range 280–350 g) were housed with free access to food and water within 24 h before experiments.

Rats were anesthetized with an intraperitoneal dose of 1% (w/v) sodium pentobarbital (45 mg/kg) and kept under anesthesia throughout the whole experiment. We performed the cisternal puncture according to the literature with some adaptations (Van den Berg et al., 2002). Firstly, the anesthetized animal was fixed onto a stereotaxic apparatus (Jiangwan I-C, Shanghai, China). The skin overlying the occipital bone was incised, and then the underlying muscle and tissue were dissected

bluntly so that the atlanto–occipital (a–o) membrane could be exposed. A 25 Gauge needle connected with a 5 cm PE-10 tube was punctured into the bottom part of a–o membrane. Once CSF flew into PE tube due to the inner pressure, the mucilage was used to fasten the needle. Afterwards, the rat was placed in the supine-90° position according to the literature in order to let CSF drip out of the tube by gravity. Before drug administration, a clamp was used to close the tube.

2.3. Experiment

2.3.1. Intra-cistern magna injection

By connecting a microsyringe with the CSF sampling tube, the intra-cistern magna formulation was injected into cistern magna with barbotage at the dose of 100 μl/kg. Afterwards, 40 μl CSF sample was withdrawn at following time points (15, 30, 45, 60, 90, 120, 180 min).

In order to testify the correctness of the method of calculating the clearance in CSF, ^{14}C-sucrose was injected into cistern magna at the dose of 100 μl/kg and sampled by the same method above. Since ^{14}C-sucrose can not be transported across BBB (Payne et al., 1996), the apparent volume of distribution at steady state (V_{ss}) should show the value of physiological CSF volume in brain and the clearance should be close or equal to the CSF bulk flow rate on rats.

2.3.2. Intranasal administration

A PE-10 tube connected with a microsyringe was inserted into the right nostril of rats to administer the nasal formulation at a dose of 8 mg/kg. CSF and blood sample were collected at the predetermined time points (5, 15, 30, 45, 60, 90, 120, 180 min). The blood sample of 0.25 ml was taken from the tail vein and placed into a heparinized PE conical tubes, then centrifuged for 10 min at 3000 rpm and at least 100 μl plasma was obtained. Meanwhile, 40 μl CSF sample was collected from the droplet of the tube and the tube was clamped after every sampling. Both plasma and CSF samples were stored at −20 °C.

2.3.3. Intravenous administration

A dose of 8 mg/kg was administered via the femoral vein. The plasma and CSF sample were collected by the same method as intranasal administration at different time points (2, 10, 20, 40, 60, 90, 120, 180 min).

2.3.4. Analytical procedures

The CSF and blood sample of MEP were analyzed by the same HPLC system only with different predisposal. After thawing, CSF sample was centrifuged at 10,000 rpm for 5 min and then analyzed directly with injection volume of 20 μl. The blood sample was processed with following steps: a volume of 100 μl plasma was placed into a 0.5 ml PE conical tube; 100 μl 10% trichloroacetic acid (v/v) was added into the tube; the mixture was vortexed for 2 min and centrifuged at 8000 rpm for 10 min. Then a volume of 20 μl of the supernatant was injected into a C18 column (Diamonsil, 20 cm × 4.6 mm, 5 μm, Dikma, USA). The column temperature was maintained at 50 °C. The mobile phase was a mixture of 50 mmol/l ammonium acetate buffer containing 2‰ triethylamine (pH = 6.0) and methanol (67:33, v/v) at a flow rate of 1.5 ml/min. The fluorescence intensity of the effluent was monitored at the excitation wavelength of 278 nm and the emission wavelength of 303 nm. The chromatographic system was composed of a LC-6A solvent delivery system, a RF-10A XL fluorescence HPLC monitor (Shimadzu, Kyoto, Japan), and a HS 2000G chromatographic integrator (HS Empire, Hangzhou, China).

The HPLC method applied above was rapid and sensitive for measuring MEP in rat CSF and plasma. The limit of quantitation of MEP was found to be 0.1 ng, which corresponded to a concentration of 5 ng/ml for CSF and 10 ng/ml for plasma with an injection volume of 20 μl. The CSF sample of ^{14}C-sucrose was analyzed by liquid scintillation counter (LKB 1210 ULTRABETA, Finland). Before counting, 3.0 ml of scintillator (30% Triton X-100, 70% xylene and 7‰ PPO) was added to CSF sample and mixed. The limit of quantitation of ^{14}C-sucrose was found to be 0.01 μCi/ml.

2.3.5. Data analysis

2.3.5.1. Calculation of drug clearance. Results of intra-cistern magna injection and intravenous administration were plotted as drug concentration–time curves. According to statistical moment, clearance of ^{14}C-sucrose and MEP in CSF/plasma were calculated as the reciprocal of the zeroth moment of a CSF/plasma level-time curve normalized for dose, $Cl = X_0/AUC$. Meanwhile, the apparent volume of distribution at steady state (V_{ss}) was also measured by the product of clearance and mean residence time (MRT), which can be obtained by the ratio of first to

zeroth moment of the drug concentration–time curve, $V_{ss} = Cl \times MRT = Cl \times AUMC/AUC$. The AUMC and AUC can be calculated by trapezoidal method as follows: $AUMC = \int_0^\infty C\,dt$ and $AUC = \int_0^\infty C\,dt$, respectively.

2.3.5.2. Calculation of the extent of drug transported to CSF. The extent of drug transported into CSF can be defined as the ratio of the total drug amount transported into CSF to the drug dose. The drug quantity transported into CSF at the time (X_A) equals the sum of drug quantity in CSF (X) and the drug quantity eliminated from CSF (X_E):

$$X_A = X + X_E \tag{1}$$

The differential equation of (1) applies:

$$\frac{dX_A}{dt} = \frac{dX}{dt} + \frac{dX_E}{dt} \tag{2}$$

According to the linear pharmacokinetic process,

$$\frac{dX_E}{dt} = k_{CSF}X \tag{3}$$

Substitution for dX_E/dt according to (3) yields:

$$\frac{dX_A}{dt} = \frac{dX}{dt} + k_{CSF}X \tag{4}$$

Because $X = V_{CSF}C$, then (4) can be changed to:

$$\frac{dX_A}{dt} = V_{CSF}\frac{dC}{dt} + k_{CSF}V_{CSF}C \tag{5}$$

Since $Cl_{CSF} = k_{CSF}V_{CSF}$, integrating (5) from zero to time (t):

$$(X_A)_t = V_{CSF}C_t + Cl_{CSF}\int_0^t C\,dt \tag{6}$$

The C_t is the drug concentration at the time (t) and the $\int_0^t C\,dt$ is the area under curve from zero to time (t). Integration of (5) from zero to infinity yields:

$$(X_A)_\infty = Cl_{CSF}\int_0^\infty C\,dt \tag{7}$$

The $(X_A)_\infty$ is the total drug quantity transported into CSF and $\int_0^\infty C\,dt$ is the area under curve from zero to infinity($AUC_{0-\infty}$).

Then, the extent of drug transported into CSF can be calculated as follows:

$$\text{extent}\,(\%) = \frac{(X_A)_\infty}{X_0} = Cl_{CSF}\int_0^\infty \frac{C\,dt}{X_0} = \frac{Cl_{CSF}(AUC_{0-\infty})_{CSF}}{X_0} \tag{8}$$

2.3.5.3. Calculation of the extent of drug transported to plasma. The extent of drug transported into plasma can be calculated using the same method as that in CSF mentioned above:

$$\text{extent}\,(\%) = \frac{(X_A)_\infty}{X_0} = Cl_{plasma}\int_0^\infty \frac{C\,dt}{X_0} = \frac{Cl_{plasma}(AUC_{0-\infty})_{plasma}}{X_0} \tag{9}$$

The drug amount was measured by multiplying the dose with the extent. One-way ANOVA was used to study the statistical difference and a value of $P < 0.05$ was considered statistically significant. Results are presented as mean values ± S.D. In addition, since MEP was reported to accord with the linear process (Holmes and Ward, 1985), the drug could be used to calculate the extent and drug amount transported by the formula above.

3. Results

The ^{14}C-sucrose concentration–time curve after intra-cistern magna injection can be seen in Fig. 1. The drug concentration–time profile of MEP in CSF and plasma after intra-cistern magna injection and intravenous administration were illustrated in Figs. 2 and 3 and the result of calculation of pharmacokinetic parameters by statistical moment can be seen in Table 1. It was found out that the Cl and V_{ss} of ^{14}C-sucrose in CSF were 0.0053 ± 0.00047 ml/min and 0.26 ± 0.041 ml, respectively, which were both close to the reported values (Kawakami et al., 1994). It indicated the intra-cistern magna injection was feasible and effective for calculating the Cl and V_{ss} in CSF. The Cl and V_{ss} of MEP in CSF were both larger than that of ^{14}C-sucrose, which were 0.047 ± 0.0027 ml/min and 0.97 ± 0.11 ml, respectively.

Table 1
Pharmacokinetic parameters of ^{14}C-sucrose and MEP in CSF and plasma

	CSF (^{14}C-sucrose)	CSF (MEP)	Plasma (MEP)
Cl (ml/min)	0.0053 ± 0.00047	0.047 ± 0.0027	24.58 ± 12.72
V_{ss} (ml)	0.26 ± 0.041	0.97 ± 0.11	5013.14 ± 1989.14

Data represent the mean ± S.D. ^{14}C-sucrose in CSF (n = 3); MEP in CSF (n = 6); MEP in plasma (n = 5).

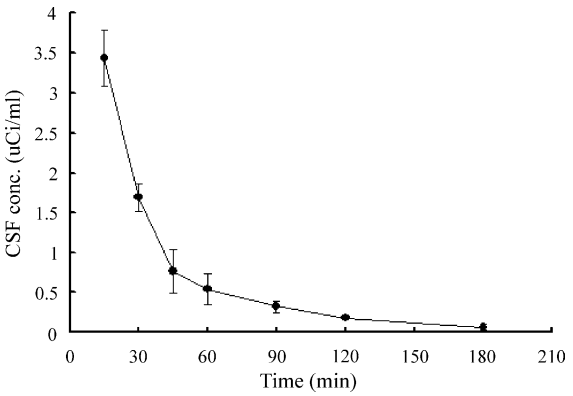

Fig. 1. Time profile of ^{14}C-sucrose concentration in CSF after intra-cistern magna injection (●). ^{14}C-sucrose concentration (μCi/ml) in CSF following intra-cistern magna injection. Mean ± S.D. (n = 3).

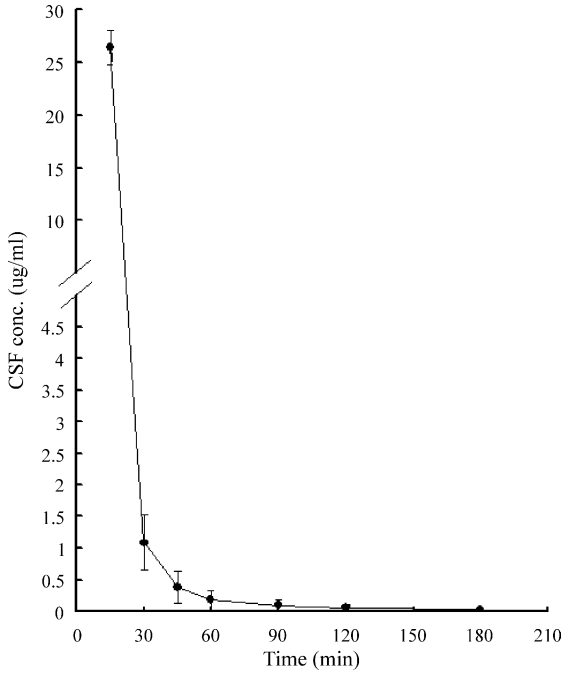

Fig. 2. Time profile of MEP concentration in CSF after intra-cistern magna injection (●). MEP concentration (μg/ml) in CSF following intra-cistern magna injection. Mean ± S.D. (n = 6).

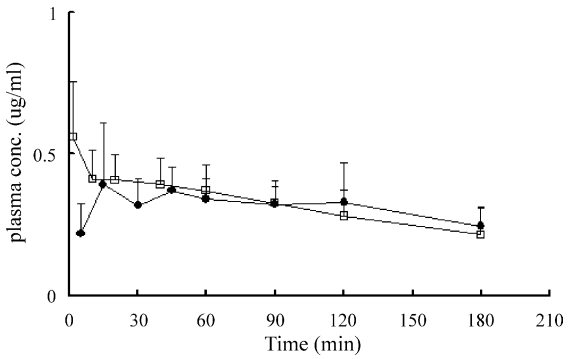

Fig. 3. Time profile of MEP concentration in plasma after intranasal (●) and intravenous (□) administration. The dose was both 8 mg/kg. MEP concentration (μg/ml) in plasma following intravenous and intranasal administration. Mean ± S.D. (n = 5).

The time profiles of MEP concentration in plasma after intranasal and intravenous administrations are shown in Fig. 3. The absorption of MEP from nasal cavity to systemic circulation was rapid and complete. The maximum concentration was achieved at 15 min after administration. The mean values of AUC after intravenous and intranasal administration were 105.44 ± 15.72 and 105.02 ± 12.74 μg min/ml. Consequently, the absolute bioavailability was nearly complete, F = 99.60% (Table 2). And after 45 min, the two curves were coincidently similar.

At 2 min following intravenous administration, the CSF level of MEP attained a high concentration of 2.71 ± 0.96 μg/ml and then was followed by an

Table 2
AUC values of MEP following intranasal and intravenous administration

Route	AUC_{Plasma} (μg min/ml)	AUC_{CSF} (μg min/ml)
Intranasal	105.02 ± 24.83	96.95 ± 31.62
Intravenous	105.44 ± 36.15	79.64 ± 18.71
Availability	99.60%[a]	121.74%[b]

[a] Absolute bioavailability.
[b] Apparent CSF availability.

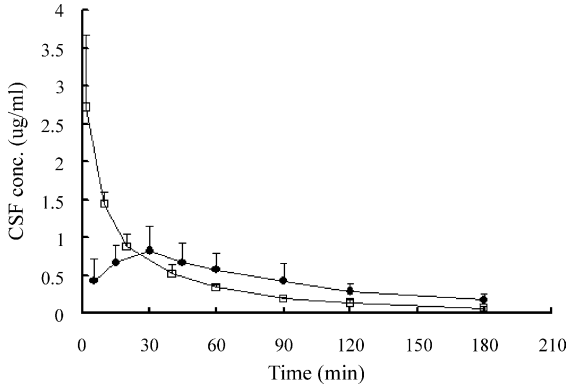

Fig. 4. Time profile of MEP concentration in CSF after intranasal (●) and intravenous (□) administration. The dose was both 8 mg/kg. MEP concentration (μg/ml) in CSF following intravenous and intranasal administration. Mean ± S.D. ($n = 5$).

exponential decline depending on the time (Fig. 4). Following intranasal administration, the transport of MEP to CSF was slow, in which the maximum concentration appeared at 30 min (Fig. 4). But because of the prolonged duration of the concentration from 30 to 180 min, the CSF level was insignificantly different from that after intravenous administration. The apparent CSF availability was 121.74% (Table 2).

The extent of MEP transported into CSF was significantly less than that into plasma after these two administrations (Table 3). It indicated that nearly all MEP was absorbed into plasma after intranasal administration and only a limited amount was transported into CSF. The drug amount transported were 2239.74 μg for plasma and 4.26 μg for CSF, respectively. As for intravenous administration, plasma also accounted for the almost total quantity at 2240.41 μg compared with 3.59 μg for CSF. By comparison between the extent and drug amount transported into CSF following intranasal administration and that after intravenous administration, no statistical difference was found, which demonstrated that there was no evidence of the existence of olfactory pathway for intranasal MEP.

Table 3
The extent of MEP transported to CSF and plasma by intranasal and intravenous administration

Extent (%)	Intravenous	Intranasal
Plasma	100.43 ± 34.44	100.30 ± 23.65
CSF	0.16 ± 0.034	0.19 ± 0.058

Data represent the mean ± S.D. ($n = 5$).

4. Discussion

Among the traditional methods evaluating the brain-targeting, comparison of the ratio of AUC after different administrations is widely used. As a matter of fact, the ratio of extent in our experiment was equal to the ratio of AUC due to the fact that the clearance of a drug is invariable as far as the same targeted region is concerned, which can be seen from Eqs. (8) and (9). Comparing with AUC values, the method not only provides a novel method to evaluate the brain-targeting, but also presents the information of drug amount in brain after administration.

In the method we applied above, the calculation of drug clearance in CSF was essential. A certain volume of ^{14}C-sucrose and MEP were injected into cistern magna and statistical moment was used to calculate the drug clearance in CSF on rats. In literatures, Muraszko et al. (1993) and Hirsch et al. (1991) employed the similar method to measure the clearance of drugs on monkey and human, respectively. They both mentioned the drug should be given in a small volume so that the total volume of the physiological CSF would not be changed too much. In our experiment, the volume administered into rat cistern magna only accounted for 10% of the total volume of CSF. And the collected CSF sample about 320 μl during 3 h experiment can be totally replaced by the CSF turnover rate of rat about 2.2 μl/min (Cserr, 1965). Therefore, the CSF serial sampling method should have little effect on the drug distribution in CSF. Additionally, the V_{ss} of ^{14}C-sucrose calculated by the same method was very close to the rat physiological CSF volume in brain about 0.2 ml (Kawakami et al., 1994), which also indicated that the method applied was equally feasible on rats. The higher clearance of ^{14}C-sucrose compared with the CSF bulk flow rate about 2.2 μl/min using extracelluar marker of inulin could be attributed to the diffusion loss of the smaller molecular weight of sucrose into brain tissue and/or trans-capillary permeation (Muraszko et al., 1993). And the anesthetized condition of rats could also play a role in affecting the reported clearance.

The clearance of MEP from rat CSF averaged 47.49 μl/min. And a quick clearance from 15 to

30 min after intra-cistern magna injection of MEP could be found (Fig. 2). Both of them illuminated that the bulk CSF flow (2.2 μl/min) only accounted for a small part of the clearance of MEP. The other part probably represented drug loss to brain tissue or across capillaries. This could be further confirmed by the calculated V_{ss} of 969.31 μl, which was nearly four times of the calculated V_{ss} of ^{14}C-sucrose. These results could be attributed to its lipophilic properties ($\log P = 2.70$) (Franklin, 1988), which made MEP easily diffuse into the brain tissue.

It can be seen in Table 3 that the extent of MEP absorbed into plasma after intranasal administration was $100.30 \pm 23.65\%$, which can be confirmed by its absolute bioavailability of 99.60%. This was also consistent with some small lipophilic compounds such as diazepam and propranolol, which had a rapid and complete systemic absorption after nasal administration (Einer-Jensen and Larsen, 2000a, 2000b). On account of the fact that oral administration of MEP often leads to low bioavailability due to high first-pass effect (Holmes and Ward, 1985), the intranasal administration suggests a promising alternative route to oral administration of MEP.

Since the transport from nasal cavity to CSF was no higher than that after intravenous administration (Table 3 and Fig. 4), there was no evidence that MEP can be transported to brain via olfactory mucosa. Similar results were shown with (S)-UH-301 and NXX-066 (Dahlin and Bjork, 2000, 2001). The t_{max} (15 min) in plasma was quicker than that (30 min) in CSF, which suggested that MEP was transported firstly through the respiratory mucosa into systemic circulation and then a part of them crossed BBB into CSF because of its lipophilic properties. These results further support the opinion (Illum, 2000) that the direct pathway from nose to brain may only be significant for compounds which are poorly absorbed from the nasal cavity to the systemic circulation or have low BBB transport properties.

In this paper, an adapted method was applied to collect CSF. Because the cistern magna is close to the essential parts of CNS such as medulla oblongata and cerebellum, etc., it is likely to endanger the motor center of rats by forwarding the needle over the skin and muscle, even if the stereotaxic apparatus was applied to locate the position of cistern magna. Therefore, a blunt dissection was performed in our experiment until the a–o membrane was exposed. Besides, compared with the method of push–pull perfusion and cisternal puncture, the CSF serial sampling method could avoid the contamination of blood, which usually happens in the procedure of cisternal puncture, and also avoid the edema in CNS, which can influence the drug disposition during the push–pull perfusion.

Although the concentration of MEP in CSF and plasma after two administrations were not significantly different, the extent and quantity of MEP transported to CSF was significantly lower than that to plasma. And it can be seen from the Eqs. (8) and (9) that the extent of drug transported to brain can be improved by enhancing AUC through various pharmaceutical technology and skills. Therefore, research work and effort should still be made on how to enhance the total transport to CSF by pharmaceutical methods.

5. Conclusion

The nasal absorption of MEP into systemic circulation was rapid and complete, which proved an effective alternative route to oral administration. Although there was no enhanced uptake from nasal cavity to CSF after intranasal administration, the method to calculate the extent and the amount of drug transported to CSF was effective to evaluate the model administration. And it can be applied to other administrations and other brain-targeting drug delivery systems.

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