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Preparation of ergoloid mesylate submicron emulsions for enhancing nasal absorption and reducing nasal ciliotoxicity

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ABSTRACT

The aim of this investigation was to prepare ergoloid mesylate submicron emulsions (EMSEs) for enhancing nasal absorption of drug and reducing nasal ciliotoxicity. Following intranasal administrations of EMSE and ergoloid mesylate solution (EMS) and intravenous administration of EMS to rats separately at the dose of 2 mg kg^{-1}, the levels of EM in blood and the cerebrospinal fluid (CSF) were evaluated by microdialysis method. The nasal ciliotoxicity was evaluated by using in situ toad palate model. The absolute bioavailability and the AUC in the CSF following intranasal administration of EMSE (56.3 ± 5.3%, AUC_{CSF} 28,594 ± 5680 ng ml^{-1} min) were statistically higher than those after intranasal administration of EMS (47.4 ± 3.5%, AUC_{CSF} 19,870 ± 2247 ng ml^{-1} min). No significant difference was found for the value of the brain drug direct transport percentage (DTP%) or the drug targeting efficiency (DTE) between the group receiving EMSE and the group receiving EMS. In conclusion, EMSE exhibited higher nasal absorption of EM in rats and significantly lower nasal ciliotoxicity whereas no greater brain-targeting efficiency in comparison with EMS.

1. Introduction

Ergoloid mesylate (EM) is widely used as a potent anti-aging medicine in clinical practice. It is composed of the methanesulfonate salts of the three hydrogenated alkaloids, dihydroergocristine, dihydroergocornine and dihydroergocryptine, in an approximate weight ratio of 1:1:1. Our earlier study (Chen et al., 2008) demonstrated that the EM solution had a statistically significant distribution advantage to the brain via the nasal route, suggesting that nasal administration could be a promising alternative for EM because the bioavailability of EM is low by the oral route (Dominiak et al., 1988; Schran et al., 1988). However, a lot of drugs and adjuncts have toxicity to nasal mucosal tissue and mucosal cilia, so further studies are needed to evaluate the nasal ciliotoxicity of the preparation.

For emulsion formulations, it has been reported that nasal absorption of drugs after microemulsion administration is fairly rapid (Li et al., 2002). The absorption of drug and brain-targeting efficiency after intranasal administration of microemulsion are significantly higher than those after administrating solution formulations (Vyas et al., 2005, 2006a,b; Jogani et al., 2008). However, report (Kawakami et al., 2002) revealed that microemulsion formulations gave damage to the gastrointestinal mucosa to some extent because it contains a relatively large amount of surfactants. Similarly, nasal ciliotoxicity of different microemulsion formulations was evaluated and some of them showed a certain irritation (Zhang et al., 2004).

Egg lecithin, as a relatively non-toxic substance, was used in most intravenous lipid emulsions. In this study, EMSE was prepared using egg lecithin as the main emulsifier and the total amount of surfactants used in EMSE was less than 5% (w/w) which was obviously less than that used in microemulsion aiming to reduce nasal ciliotoxicity. On the basis of our earlier study (Chen et al., 2008), the microdialysis method was adopted again to measure EM levels in CSF. The bioavailability and brain-targeting of EM after intranasal administration of EMSE were evaluated by comparing those after intranasal administration of EMS to rats. The nasal ciliotoxicity of EMSE and EMS was evaluated by using in situ toad palate model (Jiang et al., 1995; Zhang et al., 2004).

2. Materials and methods

2.1. Materials

EM was purchased from Guangyi Pharmaceutical Factory, China. Polyvinylpyrrolidone (PVP) was obtained from BASF Corporation, Germany. Egg lecithin (Lipoid E80®) and medium-chain triglyceride (MCT) were purchased from Lipoid KG (Ludwigshafen, Germany). Tween80 was obtained from Shenyu Medicine and Chemical Industry Limited Co. (Shanghai, China). Glycerol was supplied by Zhejiang

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Suichang Glycerol Plant (Zhejiang, China). Ephedrine hydrochloride was obtained from Apeloa Kangyu Pharmaceutical Limited Co. (Zhejiang, China). Sodium deoxycholate was supplied by Shanghai Pufei Bio-Technology Limited Co. (Shanghai, China). All other reagents were of analytical grade or the highest grade commercially available. Microdialysis probes and artificial CSF were the same as described in our earlier study (Chen et al., 2008).

2.2. Preparation of EM solution (EMS) for nasal administration, EM submicron emulsions (EMSEs) for nasal administration and EM injection

The EMS for nasal administration was prepared by dissolving PVP (2%, w/w) in water, then adding glycerol (2.5%, w/w) EDTA-2Na (0.05%, w/w), benzalkonium bromide (0.01%, w/w) and EM (0.5%, w/w). After stirring to obtain a clear solution, the pH was adjusted to about 5.0 with 0.1 mol/l HCl or NaOH solution and a 5 mg/ml EM solution for nasal administration was obtained.

MCT (20%, w/w), egg lecithin (4%, w/w) and EM (0.5%, w/w) were mixed under stirring at 80 °C to prepare the oil phase, while the glycerol (2.5%, w/w), Tween80 (0.4%, w/w), EDTA-2Na (0.05%, w/w) and benzalkonium bromide (0.01%, w/w) were dispersed in water and stirred at 75 °C to obtain the aqueous phase. The oil phase was added to the aqueous phase with continuous stirring using a high-shear mixer (ULTRA TURRAX® T18 basic, IKA® WORKS Guangzhou, Germany) at 14,000 rpm for 10 min and the primary emulsion was obtained. After adjusting the pH to about 5.0 with 0.1 mol/l HCl or NaOH solution, the primary emulsion was passed through a high pressure homogenizer (Niro Soavi NS10012k, Niro Soavi S.p.A., Via M. Da Erba, Italy) and, finally, the preparation was gassed with N_2 and sealed in 10 ml glass bottles to give a 5 mg/ml EMSE for nasal administration.

A 5 mg/ml EM injection was prepared by simply dissolving EM in double distilled water with a sufficient quantity of glycerol.

2.3. Physicochemical properties of EMSE

2.3.1. Particle size and zeta-potential

The particle size and zeta-potential were measured using a NICOMPTM 380 Zeta Potential/Particle Sizer (Particle Sizing Systems, Santa Barbara, USA). The mean particle size and distribution were measured using photon correlation spectroscopy (PCS, dynamic light scattering (DLS)), which is a powerful and versatile tool for estimating the particle size distribution of fine-particle materials ranging from a few nanometers to several micrometers (Komatsu et al., 1995). The zeta-potential was determined based on an electrophoretic light scattering (ELS) technique.

2.3.2. Drug content and entrapment efficiency of EMSE

The EM content of the formulations was determined using a high performance liquid chromatographic method at a wavelength of 280 nm. A HiQ sil C18V column (5 μm, 4.6 mm × 250 mm KYA TECH Corporation) was used for the separation of EM and a mixture of acetonitrile, water and triethylamine (45:55:1.5) was used as the mobile phase. The mobile phase was degassed and pumped isocratically at a flow rate of 1 ml/min. The injection volume was 20 μl. EM could be completely separated from the other components by this HPLC method. The linear range of EM was 30.0–300.0 μg/ml and inter-day and intra-day variations were less than 0.65% and 0.82%, respectively. The average recovery was 98.92% and the RSD was 0.25%.

The ultrafiltration method (Liu et al., 2008; Zurowska-Pryczkowska et al., 1999) was used to determine the entrapment efficiency of EMSE. Briefly, ultrafiltration was performed using a Vivapin 4 apparatus (provided by Beijing Genosys Tech-Trading Co., Ltd., Beijing, China) at 3000 rpm for 30 min. The equipment consisted of a filter membrane with a molecular weight cut-off of approximately 10 kDa. The centrifuged free drug in the aqueous phase was determined by the HPLC method described above. Then, the entrapment efficiency was calculated by comparing the drug concentration in the aqueous phase with that in the whole EMSE.

2.3.3. Measurement of viscosities of EMSE and EMS

A higher viscosity of the formulation increases the contact time between the drug and the nasal mucosa, thereby increasing the time for permeation (Arora et al., 2002). Consequently, it was necessary to measure the viscosities of EMSE and EMS for nasal administration.

After EMSE and EMS were heated to 45 °C, the samples were placed in an ice-water bath. NDJ-7 caplastometer (Shenzhen Sanli Chemicals Co., Ltd., Shenzhen, China) was used to determine the viscosities when the temperatures of the samples decreased from 45 to 5 °C. Viscosities of the two dosage forms at different temperatures were recorded.

2.4. Animal experiments

Male Sprague–Dawley rats weighing 250–300 g (animal house, Shenyang Pharmaceutical University, China) were allowed to acclimatize in environmentally controlled quarters (24 ± 1 °C and a 12:12 h light–dark cycle) for at least 5 days before the experiments. Eighteen Sprague–Dawley rats were randomly divided into three groups (n = 6/group). Two groups received EMSE or EMS intranasally, and the other group received EM solution intravenously. The experimental procedures complied with the University Animal Ethics Committee Guidelines.

2.4.1. Operations on rats before drug administration

The detailed operations were described in our earlier report (Chen et al., 2008). Briefly, a midline incision of approximately 2 cm was made parallel to the sagittal suture after the rats were anesthetized. A cranial burr hole was drilled by a dental drill to a depth of 3.1 mm and the hole was 1.5 mm lateral and 0.9 mm posterior to the bregma. The microdialysis probe was stereotaxically inserted through the cranial burr hole and was attached to the skull with dental cement. The nasal cavity was isolated from the respiratory and gastrointestinal tracts using a procedure described in literatures (Hirai et al., 1981; Huang et al., 1985). A polyethylene tube was inserted into the jugular vein for intravenous injection and blood sampling. The inflow to the microdialysis probe was driven by a microinjection pump (S200, KD Scientific Company, USA) perfused with artificial CSF, and the outflow was collected in small polypropylene tubes.

2.4.2. Drug administration and collection of biological samples

For intranasal administration, EMSE or EMS was instilled into the nostrils of rats at a dose of 2 mg kg^{-1} via a microsyringe which was attached to a blunt needle. Intravenous administration was carried out by injecting 0.1 ml of the intravenous EM solution at a dose of 2 mg kg^{-1} through an indwelling jugular vein cannula. The method of collection of the biological samples was the same as that described in our earlier report (Chen et al., 2008).

2.5. Analysis of EM in plasma and CSF

The pre-treatment of the plasma samples and RP-HPLC fluorescence analysis of EM in rat plasma and CSF samples were described in our earlier report (Chen et al., 2008).

2.6. Data analysis and statistics

The absolute concentrations in the cerebrospinal fluid (CSF) were calculated from the concentrations in the dialysates (C_d) using the following equation: $C = C_d/R$, where R is the in vivo recovery which was determined as described in our earlier report (Chen et al., 2008).

The area under the concentration–time curve (AUC) was calculated using the trapezoidal rule and the absolute bioavailability was calculated using the equation below:

$$F = \frac{AUC_{0\rightarrow\infty(i.n)}/X_{i.n.}}{AUC_{0\rightarrow\infty(i.v.)}/X_{i.v.}} \times 100\%$$

$X_{i.n.}$ is the dose administered intranasally and $X_{i.v.}$ is the dose administered intravenously.

The degree of EM targeting to CSF after intranasal administration can be evaluated by the drug targeting efficiency (DTE) (Chow et al., 1999; Zhang et al., 2004), which can be described as the ratio of the value of AUC_{CSF}/AUC_{plasma} following intranasal administration to that following intravenous injection.

$$DTE = \frac{(AUC_{csf}/AUC_{plasma})i.n.}{(AUC_{csf}/AUC_{plasma})i.v.}$$

In order to clarify the nose–brain direct transport more clearly, an index DTP% (direct transport percentage) was adopted and derived from Eqs. (1) and (2) as mentioned below (Zhang et al., 2004).

$$DTP\% = \left\{\frac{(B_{i.n.} - Bx)}{B_{i.n.}}\right\} \times 100 \quad (1)$$

where

$$Bx = \left(\frac{B_{i.v.}}{P_{i.v.}}\right) \times P_{i.n.} \quad (2)$$

Bx is AUC_{CSF} fraction contributed by systemic circulation through the blood–brain barrier (BBB) following intranasal administration. $B_{i.v.}$ is $AUC_{0\rightarrow360}$ (CSF) following intravenous administration. $P_{i.v.}$ is $AUC_{0\rightarrow360}$ (plasma) following intravenous administration. $B_{i.n.}$ is $AUC_{0\rightarrow360}$ (CSF) following intranasal administration. $P_{i.n.}$ is $AUC_{0\rightarrow360}$ (plasma) following intranasal administration.

The unpaired Student's t-test, using the computer program SPSS version 8.0 for Windows, was used. Data were presented as mean ± SD. A value of $p < 0.05$ was considered as statistically significant.

2.7. Nasal ciliotoxicity

Nasal ciliotoxicity studies were carried out using in situ toad palate model (Jiang et al., 1995; Zhang et al., 2004). Briefly, upper palate of toad (30–40 g, ♂♀animal house, Shenyang Pharmaceutical University, China) was exposed and treated with 0.5 ml test formulations (EMSE or EMS) for 30 min, then rinsed with saline. The palate was isolated and the mucocilia was examined with a 400-fold Motic DMBA 450 microscope (MoticChina Group Co., Ltd., Beijing, China). The beat of cilia was clearly observed and the lasting time of the ciliary movement was recorded. The relative percentages of lasting time of the ciliary movement of the test formulations were calculated as the ratios of the lasting time of ciliary movement after being treated with test formulations to that being treated with physiological saline. Ephedrine hydrochloride (a no obvious nasal mucociliary toxicity drug) and sodium deoxycholate (a serious nasal mucociliary toxicity agent) were used as a negative and positive control, respectively.

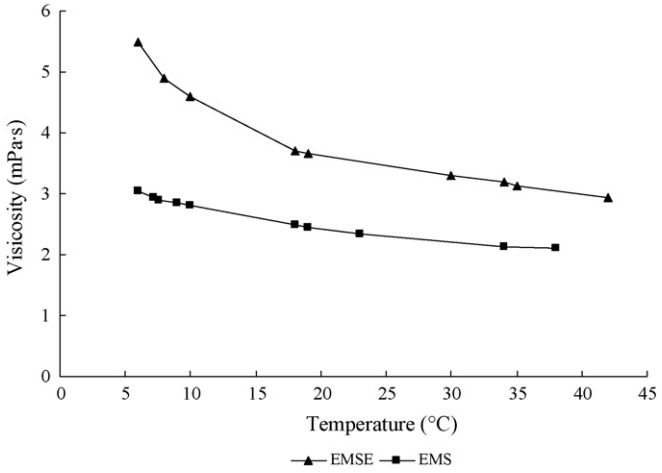

Fig. 1. Viscosity–temperature curves of EMSE and EMS.

3. Results

3.1. Physico-chemical properties of EMSE

The results showed EMSE had a mean particle size of 108.5 ± 34.3 nm, a zeta-potential of +26.2 mV. The relative percentage content and entrapment efficiency of EMSE were 100.8% and 77.2%, respectively. The results of the viscosities of EMSE and EMS are shown in Fig. 1. Obviously, the viscosities of EMSE were higher than those of EMS at different temperatures ranging from 5 to 40 °C, which indicated that the residence time of EM in the nasal cavity after EMSE administration was probably longer than that after EMS administration.

3.2. Pharmacokinetic studies

The calculated concentrations of EM in blood after intranasal and intravenous administration are shown in Fig. 2. Pharmacokinetic parameters were calculated from the observed plasma–concentration time profiles. The values of C_{max} and $t_{1/2}$ are shown in Table 1 and the values of AUC in plasma (AUC_{plasma}) are shown in Table 2. The absolute bioavailability of the group received EMSE (56.3 ± 5.3%) was statistically higher than that of the group received EMS (47.4 ± 3.5%) by nasal delivery. The time to maximum plasma concentration after EMSE administration (T_{max} = 60 min) was obviously shorter than that after EMS administration (T_{max} = 120 min). Significant differences ($p < 0.05$) were found for the values of T_{max}, AUC_{plasma} and absolute bioavailability

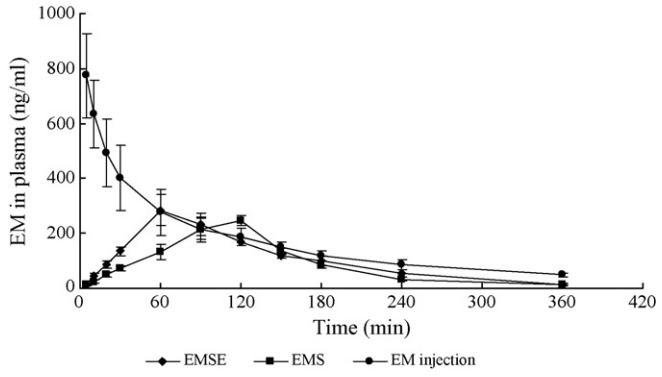

Fig. 2. Mean concentration–time curves of EM in plasma after administration of EMSE (i.n.), EMS (i.n.) and EM (i.v.) to rats (n = 6).

Table 1
Pharmacokinetic parameters of EM after administration of EMSE (i.n.), EMS (i.n.) and EM (i.v.) to rats ($n = 6$).

	Plasma		CSF	
	$t_{1/2}$ (min)	C_{max} (ng ml^{-1})	$t_{1/2}$ (min)	C_{max} (ng ml^{-1})
EMSE (i.n.)	62.60 ± 6.71	284.00 ± 57.14	264.39 ± 38.89	79.20 ± 7.53
EMS (i.n.)	58.15 ± 5.76	257.84 ± 7.92	114.74 ± 41.15	65.66 ± 2.45
EM (i.v.)	132.25 ± 12.63	774.31 ± 153.49	74.21 ± 26.08	36.98 ± 3.60

Table 2
The important parameters for evaluating brain-targeting of EM after administration of EMSE (i.n.), EMS (i.n.) and EM (i.v.) to rats ($n = 6$).

Parameter	AUC_{CSF} (ng ml^{-1} min)	AUC_{plasma} (ng ml^{-1} min)	AUC_{CSF}/AUC_{plasma} (%)	DTP (%)	DTE
EM (i.v.)	10022 ± 1089	69984 ± 9237	14.7 ± 3.6	–	–
EMSE (i.n.)	28594 ± 5680	39402 ± 3736	72.9 ± 14.5	79.1 ± 4.6	5.0 ± 1.0
EMS (i.n.)	19870 ± 2247	32768 ± 2434	60.8 ± 5.9	75.6 ± 2.7	4.1 ± 0.5

between the group received EMSE and the group received EMS whereas no significant difference was found for the values of C_{max} and $t_{1/2}$ between the two groups.

3.3. Brain-targeting study

To investigate brain-targeting of EMSE, EM levels in CSF were measured by microdialysis method following intranasal and intravenous administration to rats. The in vitro recovery from the microdialysis probe and in vivo recovery were necessary for calculating the EM concentration in the CSF, and these have been published previously (Chen et al., 2008). The calculated concentrations of EM in CSF after intranasal and intravenous administration are shown in Fig. 3, and the most important parameters are listed in Tables 1 and 2.

It was found that the EM levels in CSF following nasal administration of the two dosage forms were significantly higher than those obtained after intravenous injection. The C_{max} and AUC_{CSF} of the group received EMSE were higher than those of the group received EMS intranasally. Significant differences ($p < 0.05$) were found for $t_{1/2}$, AUC_{CSF} and C_{max} between the group received EMSE and the group received EMS intranasally.

As shown in Table 2, the ratios of AUC_{CSF}/AUC_{plasma} for groups received EMSE and EMS intranasally were 72.9 ± 14.5% and 60.8 ± 5.9%, respectively, whereas for the group received EM injection exhibited the lowest value (14.7 ± 3.6%). No significant differences were found for AUC_{CSF}/AUC_{plasma}, DTE and DTP (%) between the group received EMSE and the group received EMS intranasally.

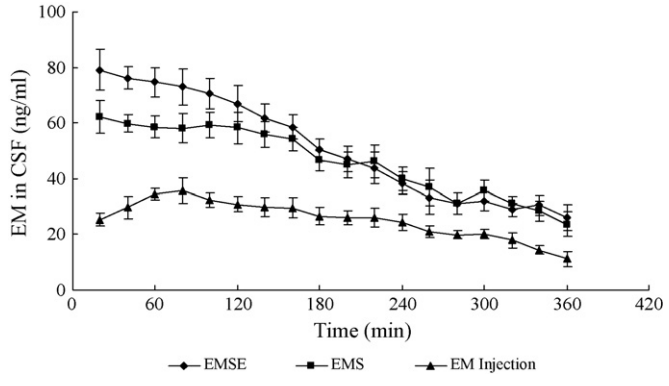

Fig. 3. Mean concentration–time curves of EM in CSF after administration of EMSE (i.n.), EMS (i.n.) and EM (i.v.) to rats ($n = 6$).

3.4. Evaluation of nasal ciliotoxicity of EMSE and EMS

It was observed that all cilia fell off from the edge of the mucosa after the upper palate of toad was treated with 1% sodium deoxycholate solution (a serious nasal mucociliary toxicity agent used as a positive control), while there were a great number of cilia with fast rate beating on the edge of mucosa after being treated with the other test formulations. The relative percentages of lasting time of the ciliary movement of the test formulations were calculated as the ratios of the lasting time of ciliary movement after being treated with test formulations to that after being treated with physiological saline and the results were shown in Table 3.

The lasting time of the ciliary movement of the group treated with EMSE was significantly shorter than that of the group treated with EMS. Significant difference ($p < 0.05$) was found for the relative percentage of lasting time of the ciliary movement between the group treated with EMSE (91.5 ± 2.7%) and the group treated with EMS (68.2 ± 2.2%). The results indicated that nasal ciliotoxicity of EMSE was lower comparing with EMS.

4. Discussion

The AUC of EM in plasma after EMSE intranasal administration was statistically higher than that after EMS intranasal administration, which suggested that nasal absorption of EM for EMSE was better than that of EMS. The reasons for absorption enhancement effect of EMSE were explained as below.

First, results of measurement of viscosities of EMSE and EMS showed viscosity of EMSE was higher than that of EMS. A higher viscosity of the formulation increases the contact time between the drug and the nasal mucosa, thereby, increasing the time for drug permeation (Arora et al., 2002).

Second, the zeta-potential of the oil droplets in emulsions plays an important role in the absorption efficiency of drug. Report (Law

Table 3
The results of the evaluation of nasal ciliotoxicity of EMSE and EMS ($n = 5$).

Samples	Lasting time of ciliary movement (min)	The relative percentage of the lasting time of ciliary movement (%)
Physiological saline	821 ± 16	100.0 ± 2.0
1% Ephedrine hydrochloride solution	813 ± 15	99.0 ± 1.8
1% Sodium deoxycholate solution	No ciliary movement was observed	–
EMSE	751 ± 22	91.5 ± 2.7
EMS	560 ± 18	68.2 ± 2.2

et al., 2001) revealed the increase of permeability of desmopressin through the nasal mucosa from positively charged liposomes was greater than that from negatively charged liposomes. The enhancement of absorption may be attributed to the bioadhesive effect of liposomes with positive charge on the negatively charged nasal mucosa (Law et al., 2001). The zeta-potential of EMSE was approximately +26 mV, consequently, the positive charged oil droplets in emulsions probably performed relatively strong interaction with the nasal mucosa and thus increase nasal absorption of EM. It was found that the zeta-potential of blank submicron emulsions containing no EM was negative. Hence, it was deduced that as a mixture of salts, EM formed cations when dissolved in water and some cations of EM existed on the surface of oil droplets and thus made the zeta-potential of EMSE positive.

Third, a wide range of absorption enhancers is known to modify transport of drugs across the nasal membrane. Enhancers, such as surfactants, bile salts, fatty acids and most phospholipids and lysophospholipids, act by modifying the phospholipid bilayer structure of cells, leaching out proteins or even stripping off the outer layer of the mucosa, thereby promoting the observed improved transcellular transport of drugs (Illum, 2002). Phospholipids probably act as absorption enhancers by a mechanism similar to that of surfactants (O'Hagan and Illum, 1990). 4% (w/w) lecithin as the main emulsifier in EMSE probably promoted the rate of intranasal transport of EM resulting in the T_{max} after EMSE administration (60 min) was obviously shorter than that after EMS administration (120 min).

As for $t_{1/2}$, the last several data points were used to estimate it. The terminal half-life is the time required for plasma concentration to decrease by 50% after pseudo-equilibrium of distribution has been reached (Toutain and Bousquet-Mélou, 2004). The half-life is a reflection of the extent of both distribution and elimination (Mehvar, 2004). The distribution is a reversible process, as the drug gets eliminated from the blood and the blood concentrations decline, the drug in the tissue will redistribute to the blood, resulting in a more sustained blood level (increased $t_{1/2}$) (Mehvar, 2004). Giving an example, when plasma concentrations of procainamide can only be detected up to 12 h post dosing, the $t_{1/2}$ based on the data points during 0–12 h period reported was between 2 and 4 h (Jamali et al., 1988). However, the $t_{1/2}$ became significant longer (8.52 ± 3.58 h) when the data points during 0–24 h period were detected using a more sensitive method (Jamali et al., 1988). It is deduced that if the collection period of the data points is not long enough to reach pseudo-equilibrium of distribution, the $t_{1/2}$ calculated might be shorter. It was reported that the elimination half-life for ergoloid in plasma was 2–5 h following extravascular administration when to data points during 0–16 h period was detected (Schran et al., 1988), and the collection period in this report was much longer than the collection period from 0 to 6 h in this study. Drug can be injected directly to blood following intravenous administration, while by the intranasal route the absorption process made the time for drug getting into blood prolonged. The time to reach pseudo-equilibrium of distribution after intravenous administration might be shorter than that by the intranasal route. Hence, it was very likely that the collection period of the data points from 0 to 6 h was not long enough to predict the half-life exactly by the intranasal route but relatively long enough by the intravenous route in this study. As a result, $t_{1/2}$ of EM in plasma after intravenous administration calculated was significantly longer than that after intranasal administration of EMSE or EMS (approximately 2 h vs 1 h). Similarly, the initial EM concentrations in CSF after intranasal administration were significantly higher than those following intravenous administration. Obviously, EM could be transferred into the CSF more quickly by the nasal route comparing with the intravenous route, so $t_{1/2}$ in CSF after intravenous administration was much shorter than that after intranasal administration of EMSE or EMS.

After nasal administration, drug can be absorbed into the systemic circulation and subsequently reaches the brain by crossing the BBB. In addition, drug can also directly travel from the nasal cavity to the CSF and brain tissue through the olfactory pathway and the trigeminal neural pathway (Thorne et al., 2004; Illum, 2000). The amount of drug absorbed via the different pathways has been shown to be highly dependent upon the characteristics of the drug and the drug formulation also plays a role (Sakane et al., 1991, 1995). The ratio of AUC_{CSF}/AUC_{plasma} following intranasal administration of EMSE or EMS was significantly higher than that after intravenous injection ($72.9 \pm 14.5\%$ for EMSE, $60.8 \pm 5.9\%$ for EMS and $14.7 \pm 3.6\%$ for EM injection), which demonstrated a statistically significant distribution advantage of EM to the brain via the nasal route. However, although AUC_{CSF} after intranasal administration of EMSE was significantly higher than that after intranasal administration of EMS, no significant difference was found for the ratios of AUC_{CSF}/AUC_{plasma} between the two groups. Obviously, comparing with EMS for the same administration route, higher AUC_{CSF} after intranasal administration of EMSE was not contributed by increase of direct nose–brain transport of EM, but rather by improvement of drug crossing the BBB because of higher absolute bioavailability of EM. Submicron emulsion as a drug carrier of EM did not exhibit higher brain-targeting efficiency than EMS by nasal delivery.

In order to more clearly understand nose–brain direct transport following nasal administration, two terms DTE and DTP (%) were introduced. The higher the DTE, the greater the degree of drug targeting to the brain can be expected after intranasal administration. DTP (%) represents the percentage of drug directly transported to the brain via the olfactory pathway and the trigeminal neural pathway by subtracting the part contributed by the systemic circulation through the BBB. It can be assumed that the AUC of the brain fraction contributed by systemic circulation through the BBB, divided by the AUC of plasma from the nasal route, is equal to that of the intravenous route (see Eqs. (1) and (2)). With the help of DTP, we could easily distinguish the EM transported directly from the nasal cavity to the brain from the apparent distribution of EM in the brain after intranasal administration. The calculated values for DTP (%) were $79.1 \pm 4.6\%$ (EMSE) and $75.6 \pm 2.7\%$ (EMS) after nasal administration, which showed that direct nose–brain transport predominated over transport through the BBB after nasal administration. As for EMSE and EMS, no significant difference was found for DTE or DTP (%) between the two groups after this two dosage forms intranasal administrated separately, which further indicated EMSE had no greater brain-targeting efficiency compared to EMS.

Brain-targeting of microemulsions of many drugs after nasal administration was studied (Vyas et al., 2005, 2006a,b; Jogani et al., 2008). When microemulsions as a drug carrier were compared to solution formulations after nasal administration, not only higher AUC in blood and brain but also higher DTE were observed, which suggested that microemulsions exhibited higher brain-targeting efficiency than solution formulations. However, in this study, submicron emulsion as a drug carrier of EM did not exhibit higher brain-targeting than EMS by nasal delivery. Comparing these two formulations, the microemulsions reported (Vyas et al., 2005, 2006a,b; Jogani et al., 2008) were prepared by using more than 40% (w/w) surfactants and cosurfactants and the particle sizes were all less than 50 nm. In this study, EMSE was prepared by using less than 5% (w/w) surfactants in order to reduce potential nasal toxicity and the particle size of EMSE was approximately 100 nm which was much larger than those of these microemulsions reported (Vyas et al., 2005, 2006a,b; Jogani et al., 2008). It is known that drugs reach the CNS from the nasal cavity by a direct transport across the olfactory region situated at the loft of the nasal cavity. The large amount of surfactants used in microemulsions acts as absorption enhancers (Muranishi, 1990; Lee et al., 1991), besides, smaller particle size can promote nasal absorption because the interfacial area is increased,

which facilitates drug release from the droplets (Kawakami et al., 2002). Therefore, it was presumed that the rate and extent of transport of drugs were improved across the olfactory region membrane and thus brain-targeting efficiency was enhanced for microemulsions.

Studies of nasal ciliotoxicity showed that 1% sodium deoxycholate solution used as a positive control caused all cilia falling off from the edge because sodium deoxycholate exerted apparently injury to nasal cilia (Wang et al., 2006). As for 1% ephedrine hydrochloride used as a negative control, there were a great number of cilia with fast rate beating on the edge of mucosa after being treated with it which indicated that it have no obvious ciliotoxicity. The relative percentage of the lasting time of ciliary movement of EMSE and EMS was $91.5 \pm 2.7\%$ and $68.2 \pm 2.2\%$, respectively. EMS exhibited a certain ciliotoxicity and it was concluded that the ciliotoxicity of EMS was mainly caused by EM because PVP added in EMS was considered non-toxic (Nguyen et al., 2006). However, ciliotoxicity was markedly reduced by preparing EM into submicron emulsions. The entrapment efficiency determined by ultrafiltration method of EMSE was 77.2%, which meant most of EM was incorporated in oil phase or located on the surface of oil droplets surface. Concentration of EM in water phase was relatively lower and less drug contacted directly with mucosa cilia, consequently, ciliotoxicity of EMSE was significant lower compared with EMS.

In conclusion, comparing with EMS, the absolute biological availability and AUC_{CSF} after nasal administration of EMSE to rats were statistically higher whereas no greater brain-targeting efficiency was observed. The advantage of nasal absorption enhancement effect of EMSE was probably caused by higher viscosity of EMSE, better bioadhesive effect caused by positive charged zeta-potential and lecithin acting as absorption enhancer. Ciliotoxicity of EMSE was remarkably reduced by reduction of the amount of EM in water phase, which make less drug contact directly with mucosa cilia.

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