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Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain

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Abstract

The purpose of this study was to improve the solubility and enhance the brain uptake of nimodipine (NM) in an o/w microemulsion, which was suitable for intranasal delivery. Three microemulsion systems stabilized by the nonionic surfactants either Cremophor RH 40 or Labrasol, and containing a variety of oils, namely isopropyl myristate, Labrafil M 1944CS and Maisine 35-1 were developed and characterized. The nasal absorption of NM from microemulsion formulation was investigated in rats. The optimal microemulsion formulation consisted of 8% Labrafil M 1944CS, 30% Cremophor RH 40/ethanol (3:1) and water, with a maximum solubility of NM up to 6.4 mg/ml, droplet size of 30.3 ± 5.3 nm, and no ciliotoxicity. After a single intranasal administration of this preparation at a dose of 2 mg/kg, the plasma concentration peaked at 1 h and the absolute bioavailability was about 32%. The uptake of NM in the olfactory bulb from the nasal route was three folds, compared with intravenous (i.v.) injection. The ratios of AUC in brain tissues and cerebrospinal fluid to that in plasma obtained after nasal administration were significantly higher than those after i.v. administration. These results suggest that the microemulsion system is a promising approach for intranasal delivery of NM for the treatment and prevention of neurodegenerative diseases.

Keywords: Nimodipine; Microemulsion; Intranasal delivery; Solubilization; Olfactory pathway; Brain targeting

1. Introduction

Nimodipine (NM), isopropyl-2-methoxyethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate, is a dihydropyridine calcium antagonist with therapeutic indications for cerebrovascular spasm, stroke and migraine (Gelmers, 1985; Langley and Sorkin, 1989). Recently, NM has been shown to be effective in ameliorating memory degeneration and preventing senile dementia in the old age (Zhang, 1993; Pantoni et al., 2000). However, the clinical usefulness of NM is limited by its high first-pass effect in liver, which leads to low oral bioavailability (Muck et al., 1996; Blardi et al., 2002) and poor brain entry. Therefore, looking for alternative routes of administration to improve therapeutic effects is necessary.

In recent years systemic drug delivery through nasal route has received a lot of attention, because it offers some advantages including rapid absorption, avoidance of hepatic first-pass metabolism, and the preferential drug delivery to brain via the olfactory region (Behl et al., 1998; Illum, 2000). Therefore, the nasal route to the brain may provide a better alternative to oral administration of NM.

For nasal delivery of NM, a challenge existing in formulation development is the solubilization of poorly water-soluble NM. The intrinsic solubility of NM is about 5.5×10^{-6} M (2.30 μg/ml). In order

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to increase the solubility of NM, ethanol, PEG 400 or Tween have been used. However, these solvents may interfere with the effect of NM. On the other hand, a moderate nasal mucosal irritation caused by a cosolvent nasal formulation comprising 40% ethanol−30% PEG 400−30% water was observed in rats (our unpublished data). Several studies have been performed using 2-hydroxypropyl-β-cyclodextrin or diheptanoyl phosphatidylcholine as a solubilizer to prepare water-soluble dosage forms of NM (Yoshida et al., 1990; Glertlova and Ondrias, 1992). Since therapeutic dose of NM for intravenous (i.v.) administration is 0.5–2 mg (Muck et al., 1996), and the effective nasal delivery volume in human is ≤400 μl (200 μl per nostril), those formulations did not provide satisfactory solubility for nasal delivery of NM.

Microemulsion is a thermodynamically stable, isotropically clear product that has a droplet size <0.15 μm. It consists of oil phase, surfactant, cosurfactant and aqueous phase. o/w microemulsions represent a promising prospect for the development of formulations suitable for the incorporation of poorly water-soluble drugs due to the high solubilization capacity, as well as the potential for enhanced absorption. In addition, the solution-like feature of microemulsion could provide advantages such as sprayability and dose uniformity. Microemulsion has been extensively studied for transdermal, parenteral, and oral delivery of drugs (Kim et al., 1996; Gao et al., 1998). While, few studies have reported the use of microemulsion for the intranasal drug delivery (Li et al., 2002).

The purpose of this study was to formulate the intranasal delivery system for NM using o/w microemulsions. The concentrations of NM in blood, the cerebrospinal fluid (CSF) and brain tissues following nasal administration of NM-loaded microemulsion were also measured, compared with i.v. administration of NM solution.

2. Materials and methods

2.1. Materials

NM was provided by Shanghai Xinyi Pharmaceutical Factory, China. NM solution (1 mg/ml) was prepared using ethanol−PEG 400−water (4:3:3) mixture. Nitrendipine was obtained from Sandong Xinhua Pharmaceutical Factory, China, and used as an internal standard. Isopropyl myristate (IPM) was purchased from Unichema Corp., UK. Oleoyl macrogolglycerides (Labrafil M 1944CS®), glyceryl monolinoleate (Maisine 35-1®), PEG-8 glycol caprylate (Labrasol®) and diethylene glycol monoethyl ether (Transcutol P®) were kindly gifted by Coloncon, UK. Polyoxyl 40 hydrogenated castor oil (Cremophor RH 40®) was provided by BASF, Germany. Double distilled water was purified using a Millipore Simplicity System (Millipore, Bedford, USA). All other reagents were of the highest grade commercially available.

2.2. Phase diagram preparation and microemulsion formulation

To find out appropriate components in the formation of o/w microemulsions, two safe and compatible nonionic surfactants, namely Cremophor RH 40 and Labrasol, combined with ethanol or Transcutol as cosurfactant were chosen. The oils employed in the present study were IPM, Labrafil M 1944CS and Maisine 35-1. The pseudo-ternary phase diagrams of oil, surfactant, cosurfactant, and water were constructed using water titration method to obtain the components and their concentration ranges that can result in large existence area of microemulsion.

Surfactant was blended with cosurfactant in fixed weight ratios (1:1, 2:1 and 3:1). Aliquots of each surfactant and cosurfactant mixture (S_{mix}) were then mixed with oil at room temperature (25 °C). For each phase diagram, the ratio of oil to the S_{mix} was varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (w/w). Water was added dropwise to each oil-S_{mix} mixture under vigorous stirring. After equilibrium, the samples were visually checked and determined as being clear microemulsions, or emulsions, or gels.

Once the microemulsion region was identified, the microemulsion formulations at desired component ratios were prepared with or without NM. The preparation of NM-loaded microemulsion was performed by dissolving NM powder into the oil-S_{mix} mixture, adding the required weight of water, and stirring to form a clear and transparent liquid. The resulting microemulsions were tightly sealed and stored at ambient temperature, and their physical stability was measured

by observing periodically the occurrence of phase separation.

2.3. Solubility of NM in o/w microemulsion

The solubilization capacity of selected microemulsions for NM was investigated and compared with the micellar solution of equivalent surfactant and cosurfactant concentration.

An excess amount of NM was introduced to 2 ml microemulsion or micellar solution, and the mixture was stirred for 48 h at 25 °C under light shielding. After standing for 1 day at room temperature, the undissolved drug was removed by centrifugation at 10,000 rpm for 10 min, then 0.5 ml supernatant was taken and the content of NM was quantified by spectrophotometry at 355 nm after dilution to 100 ml with ethanol.

2.4. Droplet size determination

The droplet size analysis of microemulsions with or without NM was conducted using a dynamic light scattering method employing a Zeta Potential/Particle Sizer NICOMP^{TM} 380 ZLS (PSS.NICOMP Particle Size System, Santa Barbara, USA). The influences of the ratio of surfactant to cosurfactant (S/CoS) and NM contents on the droplet size of microemulsion were evaluated.

2.5. Nasal ciliotoxicity

Nasal ciliotoxicity studies were carried out using in situ toad palate model (Jiang et al., 1995). In brief, the upper palate of toad (30–40 g, ♂ ♀ Experimental Animal Center of Fudan University, China) was exposed and treated with 0.5 ml test microemulsions containing NM at 6 mg/ml for 1 h, then rinsed with saline. The palate was dissected out and the mucocilia was examined with an optical microscope (Nikon Fx-35A, Japan). Saline and propranolol (a serious nasal mucociliary toxicity agent, 1% w/v solution) were used as a negative and positive control, respectively.

2.6. Nasal absorption and brain distribution studies

2.6.1. Animal experiment

Male Sprague–Dawley rats weighing 230–270 g (Experimental Animal Center of Fudan University, China) were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg) and kept on a heating pad to maintain the body temperature. The trachea was cannulated with a polyethylene tube (PE 200) to allow free breathing. An incision was made in the skin over the occipital bone. The first layer of muscle was cut, and the atlanto-occipital membrane was exposed. All of the incisions were covered with wet gauze. For the intranasal administration, about 30 min after operation, 35–40 μl of the nasal formulation (6.4 mg/ml NM in microemulsion) was administered via a PE 10 tube attached to a microlitre syringe inserted 1 cm into each nostril of rat at a dose of 2 mg/kg. For the i.v. administration, the NM solution was delivered (dose equivalent to 2 mg/kg) through the femoral vein and the injection volumes were between 0.46 and 0.54 ml.

At 2, 5, 10, 15, 30, 60, 120, 240 and 360 min after the dose, CSF samples were withdrawn by cisternal puncture (Dahlin and Björk, 2000). Briefly, a 30-gauge needle, attached to a polyethylene tube (PE 50) was inserted through the atlanto-occipital membrane, and 80–100 μl CSF was obtained by gentle suction using a syringe connected to the tube. If blood appeared immediately during sampling, the sample was discarded. The animals were decapitated immediately after CSF sampling and blood was collected from the trunk. Then the skull was cut open and the olfactory bulb (OB), olfactory tract, cerebrum and cerebellum were carefully excised. Each brain tissue was quickly rinsed with saline and blotted up with filter paper to get rid of blood-taint and macroscopic blood vessels as much as possible. After weighing, the brain tissue samples were homogenized with 1 volume of saline in a tissue homogenizer (Ika-Ultra-Turrax T25, Germany). Blood samples were anticoagulated with heparin and centrifuged at 6000 rpm for 10 min to obtain plasma. All samples, i.e. aliquots of plasma, CSF and brain tissues homogenates, were stored for up to 48 h in a deep freezer (−70 °C) until HPLC analysis. Measurements were made using 4–5 rats at each time point.

2.6.2. Analytical procedures

NM in plasma and brain tissue was assayed according to a modified HPLC method (Muck and Bode, 1994). To 200 μl plasma samples or 80–400 μl brain tissue homogenates, 50 μl nitrendipine (2 μg/ml, internal standard) and 0.1 ml 1 M NaOH were

added and extracted with 0.7 ml extraction solvent (*n*-hexane/diethylether = 50:50) by vortexing for 2 min. After centrifugation at 10,000 rpm for 10 min, the supernatant was transferred to a conical tube and the extraction repeated with another 0.7 ml extraction solvent. The separated organic phases were then united and evaporated to dryness, under a gentle stream of nitrogen at 50 °C. The residue was reconstituted in 100 μl mobile phase and then 50 μl was injected onto an HPLC system consisting of a LC-10AT VP solvent delivery system, SPD-10A UV spectrophotometric detector (Shimadzu, Japan), and HS 2000G chromatographic integrator (HS Empire, China).

CSF samples were centrifuged at 12,000 rpm for 20 min and 50 μl supernatant was injected into the HPLC system directly.

Chromatographic separation was achieved at ambient temperature on a 4.6 mm × 200 mm, C_{18} analytical column (Diamonsil™, Dikma) attached to a guard column (Nova-Pak, 10 μm, C_{18} 15220, Waters). For plasma and brain tissue samples, the mobile phase was 40% 0.05 M ammonium acetate/60% acetonitrile (v/v) at a flow rate of 1 ml/min and a detection wavelength of 358 nm. The retention time was 10.4 min for NM and 9 min for the internal standard, nitrendipine. For CSF samples, the mobile phase was a mixture of 35% 0.05 M ammonium acetate/65% acetonitrile (v/v) at a flow rate of 1 ml/min. NM was detected at 238 nm with a retention time of 7.8 min.

Calibration curves of NM were prepared with plasma and respective tissues spiked with known amounts of the drug utilizing its HPLC peak area ratio to the internal standard. Standard and control samples for the CSF analysis were made from artificial CSF (Chou and Donovan, 1997) and the peak area was used for quantification of NM. The linear range of NM was 15–1000 ng/ml, 15–4000 ng/g and 3–100 ng/ml and inter- and intra-day variations were less than 6, 4 and 5% for plasma, brain tissue, and CSF samples, respectively. The extraction recoveries of NM from plasma and tissue homogenates were more than 90 and 80%, respectively. The detection limits were 10 ng/ml (or 10 ng/g) for plasma and tissue samples, and 2 ng/ml for CSF samples.

As NM is sensitive to light-induced degradation, all operations except data evaluation were carried out under thorough light shelter (Muck and Bode, 1994).

2.6.3. Data analysis

All concentration data were dose- and weight-normalized. The C_{max} following i.v. injection was estimated by fitting the plasma concentration data to a conventional two-compartment model using pharmacokinetics analysis program. The C_{max} and t_{max} values of the nasal administration were read directly from the concentration–time profile. The area under the concentration–time curve ($AUC_{0\to t}$) was calculated by the trapezoidal rule. The variance for the $AUC_{0\to t}$ was estimated by the method of Yuan (1993). The absolute nasal bioavailability of NM from microemulsion was calculated as the ratio of $AUC_{i.n.}$:$AUC_{i.v.}$.

To evaluate the brain targeting after nasal dosing, two indexes were adopted:

(1) Drug targeting efficiency (DTE) (Chow et al., 1999; Liang et al., 2003). DTE represents a time-average partitioning ratio.

$$\mathrm{DTE} = \frac{\mathrm{AUC_{brain}}}{\mathrm{AUC_{plasma}}}$$

(2) In order to clarify nose–brain direct transport more clearly, we introduced a term of 'brain drug direct transport percentage (DTP)', which was defined based on Eqs. (1) and (2):

Hypothesis:

$$\frac{B_{iv}}{P_{iv}} = \frac{B_x}{P_{in}} \quad (1)$$

$$\mathrm{DTP}\,\% = \frac{B_{in} - B_x}{B_{in}} \times 100\% \quad (2)$$

where $P_{i.v.}$, $B_{i.v.}$, $P_{i.n.}$, $B_{i.n.}$, respectively, denote the $AUC_{0\to 360}$ of NM in plasma and brain tissue that obtained after i.v. and intranasal administration. B_x represents the brain AUC fraction contributed by systemic circulation through the blood−brain barrier (BBB) after nasal dosing.

It is believed that drug uptake into brain from the nasal mucosa via two different pathways. One is systemic pathway that some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing BBB. The other is olfactory pathway that partial drug can travel from the olfactory region in the nasal cavity directly into CSF and brain tissue (Illum, 2000). We can deduce that the amount of drug in the brain tissue after nasal application attributes to these two parts. Since a linear pharmacokinetic in

NM has been demonstrated (Rameis, 1993), the drug amount is proportional to AUC. Thus, we assume that the brain AUC fraction contributed by systemic circulation through BBB (represented by B_x) divided by plasma AUC from nasal route is equal to that of i.v. route (see Eq. (1)). Then DTP represents the percentage of drug directly transported to the brain via olfactory pathway.

The statistical differences between nasal and i.v. treatment were assessed using an unpaired student's *t*-test and a value of $P < 0.05$ was statistically significance.

3. Results and discussion

3.1. Phase behavior

When Cremophor RH 40 and ethanol were selected as surfactant and cosurfactant, o/w microemulsions were formed at 25 °C with the various oils tested with the exception of Maisine 35-1. However, only Maisine 35-1 produced a microemulsion system with Labrasol as a surfactant and Transcutol as a cosurfactant. To screen out a drug vehicle suitable for intranasal delivery of NM, three different microemulsion systems were examined. Phase studies were done to investigate the effect of S/CoS on the existence range of stable o/w microemulsion region.

3.1.1. IPM/Cremophor RH 40/ethanol/water system

The pseudo-ternary phase diagrams with various weight ratios of Cremophor RH 40 to ethanol were displayed in Fig. 1 with the shaded region highlighting the o/w microemulsion region. The area of microemulsion region increased with increasing ratio of S/CoS and became constant at a ratio greater than 3:1. A maximum of 9% w/w IPM was incorporated in microemulsion when using S_{mix} in a ratio of 3:1. The viscosity of microemulsion was also affected by the surfactant and ethanol content, with the higher weight percentage of Cremophor RH 40, the viscosity of the microemulsion formulation increased. A gel area was seen in the 2:1, 3:1 and 4:1 phase diagrams.

3.1.2. Labrafil M 1944CS/Cremophor RH 40/ethanol/water system

The area of microemulsion existence obtained using Labrafil M 1944CS as an oil was depicted in Fig. 2. A similar trend as above was observed in o/w microemulsions produced where the area of microemulsion region increased markedly with higher Cremophor RH 40 concentrations, and Labrafil M 1944CS could be solubilized up to 14% w/w at the ratio 3:1.

3.1.3. Maisine 35-1/Labrasol/Transcutol/water system

The results of the phase study with Labrasol as surfactant and Transcutol as cosurfactant were shown in Fig. 3. Microemulsion prepared by Labrasol, in

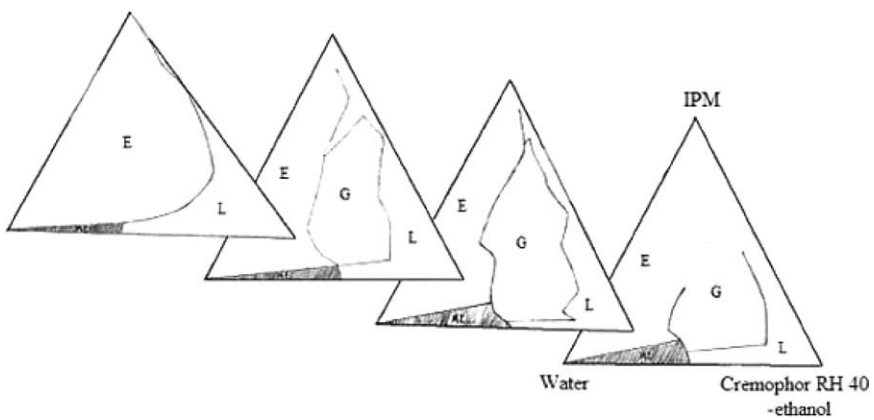

Fig. 1. Pseudo-ternary phase diagrams composed of IPM, Cremophor RH 40–ethanol (Cremophor RH 40–ethanol = 1:1, 2:1, 3:1 and 4:1, left → right) and water. E, emulsion; G, gel; L, isotropic region; shaded region (ME), o/w microemulsion.

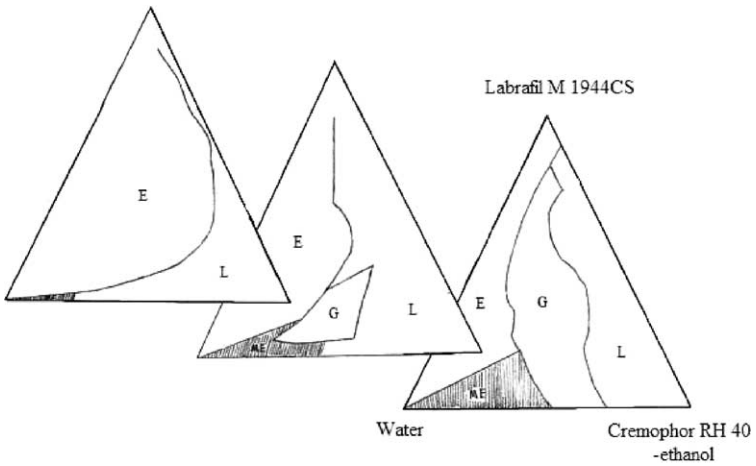

Fig. 2. Pseudo-ternary phase diagrams composed of Labrafil M 1944CS, Cremophor RH 40–ethanol (Cremophor RH 40–ethanol = 1:1, 2:1, and 3:1, left → right) and water. E, emulsion; G, gel; L, isotropic region; shaded region (ME), o/w microemulsion.

commom with those made using Cremophor RH 40, exhibited a greater microemulsion region area with increasing ratio of Labrasol to Transcutol, and approximately 12% w/w Maisine 35-1 was found to be solubilized at S/CoS of 3:1. However, no gel area was observed in these phase diagrams, so no distinct conversion from w/o to o/w microemulsion was seen.

3.2. Microemulsion composition

The phase study revealed that the maximum proportion of oil was incorporated in three different microemulsion systems at S/CoS of 3:1. From a formulation viewpoint, the increased oil content in microemulsions may provide a greater opportunity for the solubilization of NM. Moreover, the composition (%, w/w) of S_{mix} in a microemulsion preparation was less than 30%, the formulation was less viscous which could be sprayable using a pump spray device. Hence, three microemulsions were chosen from the 3:1 phase diagrams and defined as IPM, 1944 and Maisine, respectively. The detailed compositions of these microemulsion formulations were shown in Table 1. Based on visual identification, these three

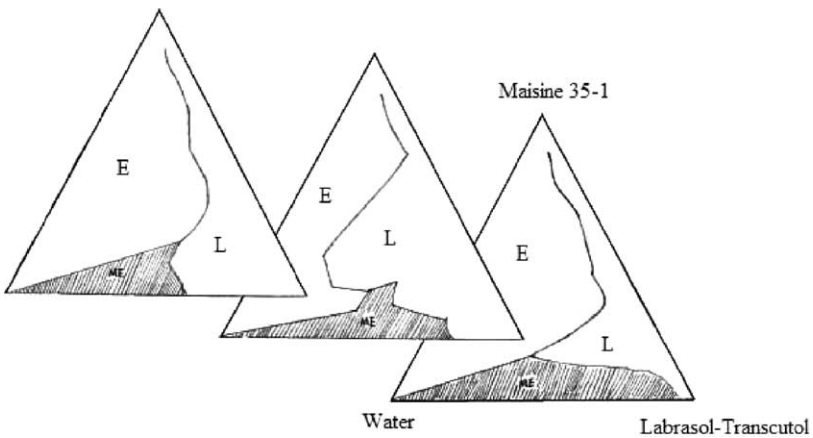

Fig. 3. Pseudo-ternary phase diagrams composed of Maisine 35-1, Labrasol–Transcutol (Labrasol–Transcutol = 1:1, 2:1, and 3:1, left → right) and water. E, emulsion; L, isotropic region; shaded region (ME), microemulsion.

Table 1
Composition of the selected microemulsion formulations

IPM	%, w/w	1944	%, w/w	Maisine	%, w/w
IPM	5	Labrafil M 1944CS	8	Maisine 35-1	7
Cremophor RH 40	22.5	Cremophor RH 40	22.5	Labrasol	22.5
Ethanol	7.5	Ethanol	7.5	Transcutol	7.5
H_2O	65	H_2O	62	H_2O	63

Table 2
Incorporation of NM into microemulsion and micelles

Formulation	Drug incorporation (mg/ml)	
	Microemulsion	Micellar system
IPM	6.19 ± 0.20[a]	3.29 ± 0.02[b]
1944	6.45 ± 0.03[a]	3.29 ± 0.02
Maisine	6.42 ± 0.20[a]	3.35 ± 0.09

[a] Significantly different from that micellar system, $P < 0.01$.
[b] Mean ± S.D. ($n = 3$).

microemulsions with or without NM remained as clear liquid for a period of 2 months without the occurrence of phase separation at the room temperature.

3.3. Solubility of NM in three microemulsions

The drug entrapment in three microemulsion formulations and the corresponding micellar solution were given in Table 2. The solubility of NM, a practically water-insoluble compound (2.3 μg/ml), was improved dramatically by the microemulsion. It reached about 6.4 mg/ml, an approximately 2783- and 2-fold increase compared with intrinsic solubility and solubility in the micellar solution (3.3 mg/ml), respectively. There was no statistic difference in solubilization for NM among the three microemulsion formulations.

It has been suggested that the existence of an extra locus for drug incorporation in a microemulsion, namely a core of oil in the center of the aggregate, further increase drug solubility, compared with a micellar system (Warisnoicharoen et al., 2000). Hence, much higher values of NM incorporation were found in the microemulsion than in the micellar solution. It is known that the drug carrying improvement using o/w microemulsion depends on the solubility of the drug in the dispersed oil phase and the percentage of that phase present (Malcolmson and Lawrence, 1993). Although NM in IPM exhibited relatively higher solubility (the solubility of NM in IPM, Labrafil M 1944CS, Maisine 35-1 was 6.70, 4.42 and 4.25 mg/g, respectively), only 5% w/w of IPM presented in microemulsion, lower than Labrafil M 1944CS or Maisine 35-1, therefore no significant difference among the entrapment of NM into these three microemulsion formulations.

3.4. Particle size

Microemulsions without NM were prepared with equivalent oil content and varying weight ratios of S/CoS. The fairly low oil content was chosen in order to ensure samples were well within the o/w microemulsion area. The results for the particle size analysis of various microemulsions were listed in Table 3. The droplet size of microemulsions containing IPM was not affected significantly by the increasing concentration of surfactant. It could be due to a

Table 3
Droplet size (nm) of the plain microemulsion with the variety of S/CoS

S/CoS	IPM–S_{mix}–water (% w/w, 2:30:68)	Labrafil M 1944CS–S_{mix}–water (% w/w, 4:30:66)	Maisine 35-1–S_{mix}–water (% w/w, 4:30:66)
1:1	26.3 ± 4.5[a]	49.3 ± 8.8	78.2 ± 8.8
2:1	23.7 ± 5.3	27.0 ± 6.1	60.5 ± 15.5
3:1	26.7 ± 4.1	26.7 ± 3.3	32.2 ± 4.7
4:1	23.8 ± 5.3	–	–

[a] Mean ± S.D.

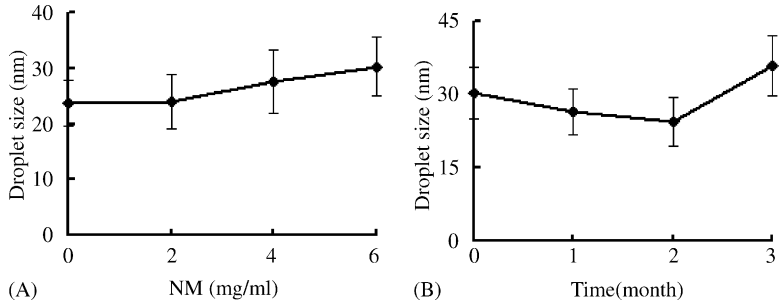

Fig. 4. (A) The droplet size of microemulsion system (Labrafil M 1944CS–Cremophor RH 40–ethanol–water = 8:22.5:7.5:62) loaded with NM from 2 to 6 mg/ml; (B) Change in mean particle size of microemulsion system containing NM at 6 mg/ml as a function of time at room temperature. Data represent the mean ± S.D.

small amount of oil (2% w/w IPM) used here. In the case of a microemulsion using Labrafil M 1944CS or Maisine 35-1 as oil, the droplet diameter decreased with increasing ratio of S/CoS. This result is in accordance with the report that the addition of surfactant to the microemulsion systems causes the interfacial film to condense and to be stable, while the cosurfactant causes the film to expand (Kale and Allen, 1989). As shown in Table 3, the mean droplet sizes of these three microemulsion systems were about the same at S/CoS of 3:1.

The effect of drug contents on the mean droplet size of NM-loaded microemulsions (Labrafil M 1944CS–Cremophor RH 40–ethanol–water = 8:22.5:7.5:62) was illustrated in Fig. 4. When NM was added at concentrations from 2 to 6 mg/ml, the microemulsion size increased slightly with increasing the NM concentration. No significant droplet size change was found when these preparations were stored at ambient condition for 3 months (Fig. 4), which indicated that NM-loaded microemulsion was physically stable.

3.5. Nasal ciliotoxicity

Mucociliary clearance contributes to the body's primary non-specific defence mechanism by propelling potentially hazardous substances such as dust, microorganisms, allergens and carcinogens towards the pharynx where they are swallowed or expectorated (Proctor, 1977). If the normal mucociliary clearance were inhibited, patients would suffer extensively from chronic respiratory infections. These consequences emphasize that the constituents of preparations intended for nasal delivery should not adversely affect the clearance system, especially in the chronic therapy. Therefore, a requirement in formulation development is no nasal mucosal irritation from the microemulsion. Optical microscopic results showed that there were a great number of cilia with fast rate beating on the edge of mucosa that was treated with 1944 microemulsion for 1 h (Fig. 5), and the same phenomenon was observed when the contact time was extended up to 4 h, indicating that 1944 microemulsion had no obvious effect on the cilia movement. While those microemulsions formulated with IPM and Maisine 35-1 had moderate irritation. It was observed that some cilia fell off from the edge of the mucosa, and the remaining was still beating, but the duration of ciliary movement decreased. The difference of ciliotoxicity between IPM and 1944 microemulsion may be related to the type of oil and the percentage of oil phase present.

Taken together, considering the solubilization capacity, droplet size and nasal ciliotoxicity, 1944 microemulsion seems to be an optimal formulation for nasal delivery of NM.

3.6. In vivo absorption and brain distribution of NM-loaded microemulsion

Although a number of substances, including viruses, metals, dyes, peptides and some therapeutic agents have been reported to gain direct access to the brain after nasal administration, little is known about the direct nose–brain transport of nasally applied NM in an o/w microemulsion. To elucidate whether the microemulsion formulation affect not only the nasal

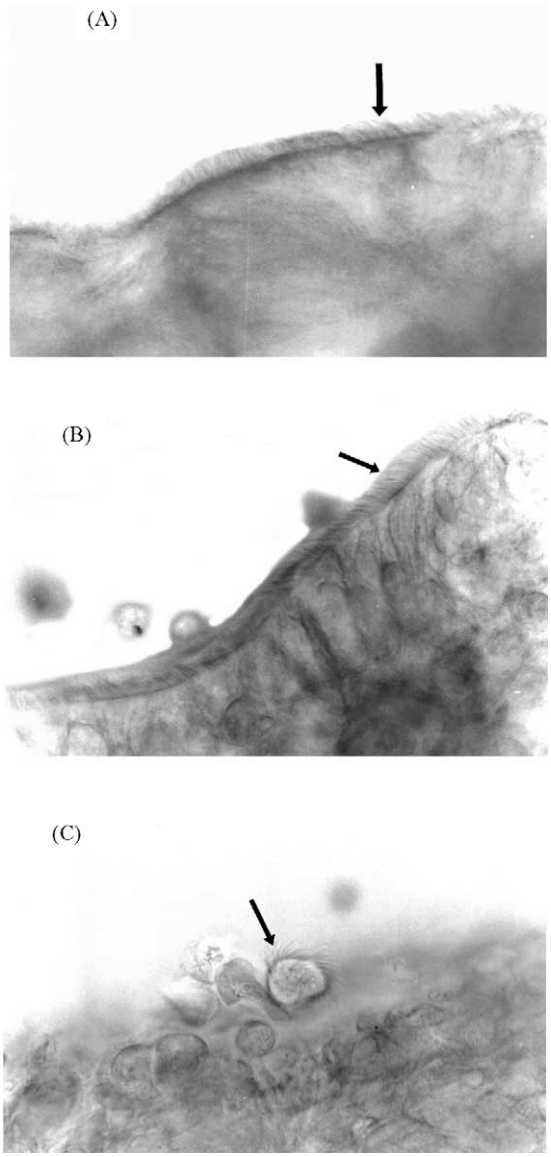

Fig. 5. Optical microscopic images of (A) negative control (saline, cilia on the mucosa was intact, dense and beat actively 1 h after treatment), (B) 1944 microemulsion (similar phenomenon with that of saline was visualized 1 h after treatment), and (C) positive control (1% propranolol solution, no cilia on the mucosa was observed but a few exfoliated cilia 5 min after treatment). Cilia are indicated by arrow (10× 40 magnification, $n = 4$–5).

absorption of NM but also its brain distribution, we determined the concentrations of NM in blood, CSF and four brain regions. Fig. 6 represents the mean plasma and brain tissue concentration–time profiles

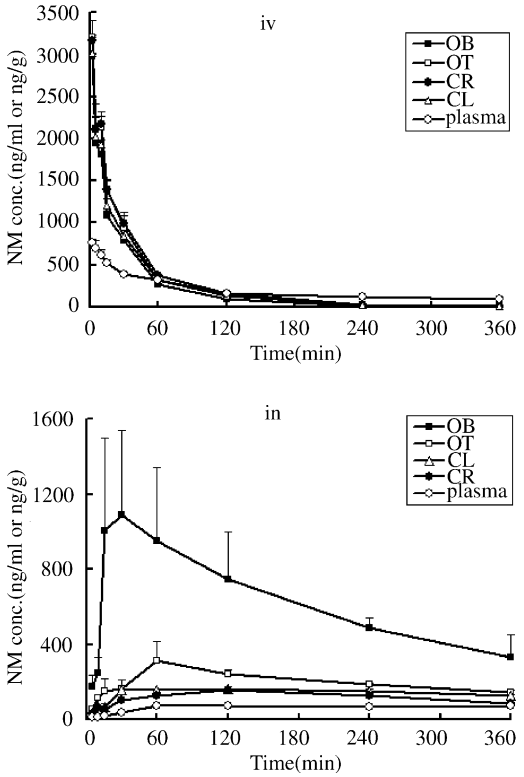

Fig. 6. Plasma and brain tissue concentration–time profiles of NM after i.v. and nasal administration of 1944 microemulsion at 2 mg/kg dose in rats. Data represent the mean ± S.E.M. i.v., intravenous NM solution; i.n., intranasal microemulsion; OB, olfactory bulb; OT, olfactory tract; CR, cerebrum; CL, cerebellum.

of NM after i.v. injection and nasal administration of 1944 microemulsion to rats at a dose of 2 mg/kg. The non-compartmental pharmacokinetic parameters were given in Table 4. Nasal absorption of NM from microemulsion preparation into systemic circulation exhibited the sustained release effect and the maximum plasma concentration was arrived at 1 h. The plasma levels remained constant for 6 h. The absolute bioavailability of microemulsion optimized in this study was 32.4%, which was markedly superior to that of oral NM solution, a bioavailability of 1.2% was found previously in rats (data not shown).

Following i.v. administration, brain NM concentrations reached peak level at 2 min and declined fast with time. Similar NM concentrations were found in different brain tissues. Following intranasal administration of 1944 microemulsion, the profiles of NM level in

brain displayed an initial absorption phase and maximum concentration achieved after about 30 min in OB and 60–120 min in other regions of the brain. NM content differed considerably in different brain regions. The highest concentration was observed in the OB (the peak drug level was 1090.2 ± 452.4 ng/g), followed by the olfactory tract, then the cerebellum and cerebrum. These findings are in good agreement with that previously reported by Chow et al. (1999) for the intranasal administration of cocaine and support the existence of a nose–brain direct pathway.

As shown in Fig. 6, NM was particularly concentrated in the OB after nasally administered microemulsion, the $AUC_{0\rightarrow360}$ value was 3.02 times (225,141 ± 38,529 ng min/g versus 74,560 ± 3617 ng min/g) that obtained after i.v. injection, this value was also considerably higher (225,141 ± 38,529 ng min/g versus 103,574 ± 6922 ng min/g) compared with intranasal NM solution in our previous study (data not shown). However, the uptakes of NM into other brain regions after nasal dosing of microemulsion were lower than those after i.v. injection (Table 4). The reason for this is not clear. There are three possibilities. First, the high initial plasma concentration after i.v. administration may have caused high, rapid transport of NM crossing the BBB by passive diffusion. Therefore, relative low plasma levels from NM-loaded microemulsion might result in a reduced distribution of NM into brain (systemic pathway). Second, in contrast to cerebrum and cerebellum, the mass of the OB is small. The distribution of NM from OB into other brain regions could be counteracted by their large masses, thus leading to the insignificant increase in NM content in other brain tissues (olfactory pathway). Third, due to the brain NM concentrations 6 h after i.v. injection nearing zero, NM levels in plasma and brain were not monitored under an extended time following both routes of administration. Since the microemulsion administered nasally displayed a slow absorption and decline, if the experiment time were prolonged (12 or 24 h) or a repeated intranasal dosing were employed, the nose–brain direct transport could be increased.

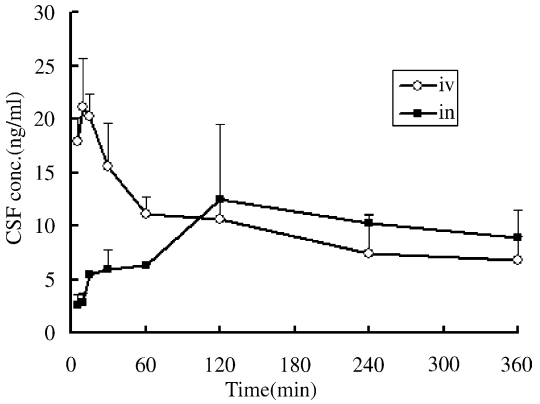

Fig. 7. CSF concentration–time profiles of NM after i.v. and nasal administration of 1944 microemulsion at 2 mg/kg dose in rats. Data represent the mean ± S.E.M. i.v., intravenous NM solution; i.n., intranasal microemulsion.

The concentration–time profile in CSF (Fig. 7) revealed no increased NM concentration after nasal administration of microemulsion compared with i.v. administration. The highest CSF concentration of NM from the microemulsion was 12.5 ± 7.0 ng/ml, lower than that of intranasal NM solution in our previous work (21.0 ± 4.8 ng/ml), suggesting that

Table 4
Pharmacokinetic parameters following i.v. and nasal administration of NM-loaded microemulsion

Parameters	Route	Plasma	CSF	OB	OT	CR	CL
C_{max} (ng/ml g)	i.n.	73.4 ± 4.4[a]	12.5 ± 7.0	1090.2 ± 452.4	315.2 ± 101.6	157.8 ± 19.3	164.4 ± 9.8
	i.v.	807.6 ± 86.9	21.1 ± 4.5	2976.2 ± 255.5	3211 ± 199.3	3164 ± 246.9	3016 ± 184.8
$AUC_{0\rightarrow360}$ (ng min/ml g)	i.n.	23185 ± 1330	3367 ± 655	225141 ± 38529	72452 ± 5359	43621 ± 3007	52227 ± 2714
	i.v.	71655 ± 2348	3604 ± 514	74560 ± 3617	91588 ± 4420	99391 ± 4217	86564 ± 3958
Ratio of $AUC_{i.n.}$/ $AUC_{i.v.}$ (%)		32.36[b]	93.42	301.96	79.11	43.89	60.33

i.v.: NM solution; i.n., NM-loaded microemulsion; OB, olfactory bulb; OT, olfactory tract; CR: cerebrum; CL, cerebellum; CSF, cerebrospinal fluid.

[a] Mean ± S.D. ($n = 4$–5).

[b] Bioavailiability.

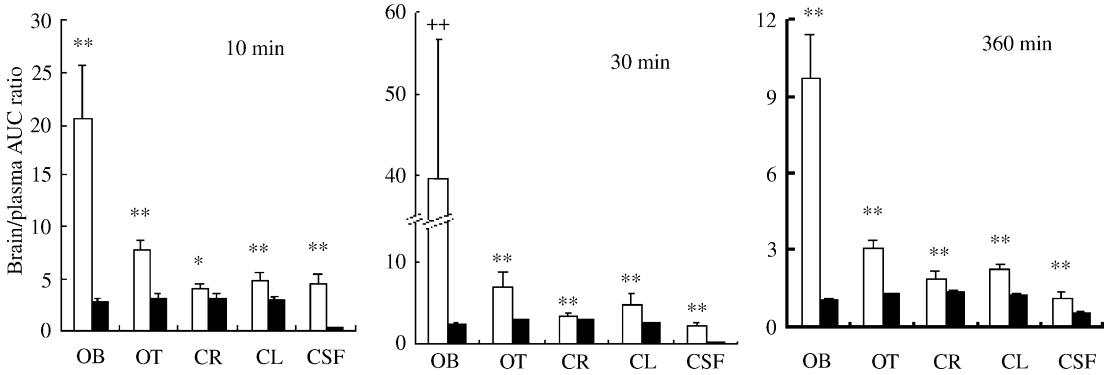

Fig. 8. AUC ratio of NM between various brain tissue and plasma at three time points after i.v. and intranasal administration to rats at a dose 2 mg/kg. Microemulsion (□); i.v. solution (■). The data of CSF-to-plasma AUC ratios from two routes presented here by multiplying 10. Data represent the mean ± S.D. OB, olfactory bulb; OT, olfactory tract; CR, cerebrum; CL, cerebellum; CSF, cerebrospinal fluid. $^{*}P < 0.05$, $^{**}P < 0.01$.

the microemulsion increase the affinity of NM with brain tissue, which resulted in NM being more easily transferred to OB than to CSF.

To allow comparison of brain NM content after i.v. and nasal delivery and evaluate the brain targeting of NM-loaded microemulsion, brain NM contents were therefore normalized by the plasma NM content of the corresponding time point and the brain-to-plasma NM AUC ratios at 10, 30, 360 min following both routes of administration were illustrated in Fig. 8. Results showed that the ratio of AUC in brain tissues and CSF to that in plasma after nasal administration of 1944 microemulsion were significantly higher ($P < 0.05$) than those after i.v. injection. For example, at 10 min after nasal dosing, the AUC ratio was 7.3 times higher than that after i.v. dosing (20.4 ± 5.2 versus 2.8 ± 0.3) in OB and about 19.2 times higher (0.46 ± 0.09 versus 0.024 ± 0.003) in CSF. Similarly, at 30 min post-administration, a 29 times increase (39.7 ± 17.0 versus 2.4 ± 0.1) in OB and about 6.9 times increase (0.22 ± 0.04 versus 0.032 ± 0.002) in CSF were observed on 1944 microemulsion dosing.

In order to more clearly present nose–brain direct transport following nasally applied microemulsion, we introduced a term of 'brain drug direct transport percentage (DTP)'. The calculated DTP values were shown in Fig. 9. Following intranasal administration, about 26–89% of NM content at 6 h were transported to the brain via the olfactory pathway. In short, these results support the existence of an alternative brain

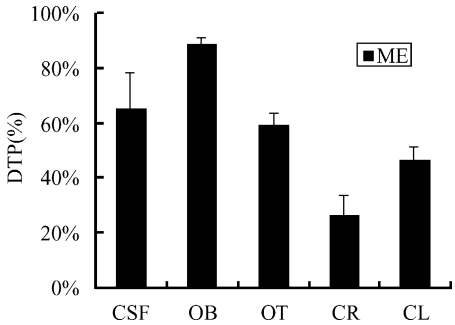

Fig. 9. Brain drug direct transport percentage (DTP) after nasal administration of NM-loaded microemulsion. Data represent the mean±S.D. ME, microemulsion; OB, olfactory bulb; OT, olfactory tract; CR, cerebrum; CL, cerebellum; CSF, cerebrospinal fluid.

entry pathway for NM by formulating in o/w microemulsion. For those CNS-active polypeptides or protein drugs which can't penetrate BBB, microemulsion intranasal delivery might enhance their brain uptake because of its protection against enzymatic hydrolysis and increasing the quantity of drug to the brain.

4. Conclusion

The microemulsion system comprising 8% Labrafil M 1944CS, 30% Cremophor RH 40/ethanol (3:1) and water was optimal for intranasal delivery of NM. This

preparation had a maximum solubility of NM up to 6.4 mg/ml with no nasal ciliotoxicity. The microemulsion system is physical stable at ambient conditions for 3 months. In vivo absorption studies revealed that the microemulsion resulted in a constant NM concentration in the blood, this is beneficial to reducing hypotensive effects induced when given as i.v. injection. Brain distribution study and DTP results confirmed that a fraction of NM could be transported directly into the brain after nasal delivery of NM-loaded microemulsion. The brain targeting might be more pronounced when NM administered chronically.

In conclusion, the microemulsion system is a promising approach for the intranasal delivery of NM for the therapeutic effects improvement. Further studies are necessary to investigate the pharmacological/toxicological consequences of direct nose–brain transport of nasally applied NM-loaded microemulsion.

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