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Research paper

Evaluation of brain-targeting for the nasal delivery of ergoloid mesylate by the microdialysis method in rats

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Abstract

The aim of this study was to quantify the nasal delivery of ergoloid mesylate (EM) to the brain by comparing cerebrospinal fluid (CSF) and plasma EM levels after nasal administration at a dose of 4 mg/kg with those after intravenous administration. Following nasal delivery, EM reached a C_{max} value (mean ± SD) in plasma of 348.41 ± 19.47 ng/ml and in CSF of 87.35 ± 6.37 ng/ml after 107 and 20 min, respectively, while after intravenous injection, EM reached a C_{max} value (mean ± S.D.) in CSF of 54.81 ± 4.92 ng/ml at 60 min and the C_{max} in plasma was 1255.51 ± 133.59 ng/ml. The AUC_{CSF}/AUC_{plasma} ratio (0.48 ± 0.05) after intranasal delivery differed greatly from the ratio (0.14 ± 0.04) observed after intravenous injection ($P < 0.05$). The further analyzed data demonstrated a statistically significant distribution advantage of EM to the brain via the nasal route, and further suggesting that nasal administration can be a promising alternative for EM that undergoes first-pass metabolism following oral administration.

Keywords: Ergoloid mesylate; Nasal delivery; Microdialysis; Brain-targeting; RP-HPLC with fluorescence detection

1. Introduction

Ergoloid mesylate (EM), the mixture of the methanesulfonate salts of the three hydrogenated alkaloids, dihydroergocristine ($C_{35}H_{41}N_5O_5 \cdot CH_4O_3S$, MW = 659.79), dihydroergocornine ($C_{31}H_{41}N_5O_5 \cdot CH_4O_3S$, MW = 707.84), and dihydroergocryptine ($C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$, MW = 673.82) in an approximate weight ratio of 1:1:1, presents itself as a remarkable anti-aging medicine and has been the most widely used in clinical practice. Several mechanisms of action within the brain, which attribute to its efficacy, were proposed [1–9], amongst others enhancing metabolism in brain cells [1,2], protecting the brain from damage [3], and interaction with neurotransmitter systems [4,5]. Many investigations [8,10–13] have claimed that this drug can improve a variety of signs and symptoms, especially senile dementia and cerebrovascular insufficiency.

The most widely used form of administration of EM is the oral route which has a low bioavailability (<10%) [14,15] resulting from incomplete absorption and first-pass effect. Although injection could avoid the drawbacks of oral therapy, the small molecular weight and lipophilic ($\log P = 2.65$) character of EM make transport across the blood–brain barrier (BBB) likely. However, additional problems such as poor patient compliance and topical painfulness will still not be solved. Therefore an alternative administration is in great need.

Intranasal administration has drawn considerable interest in the last decade since it provides a non-invasive method for bypassing hepatic first-pass effect and possibly the BBB [16–18]. Therefore, it might be a feasible way of both enhancing EM's bioavailability and achieving its brain-targeting. Drugs administered to the nasal cavity can travel along the olfactory and trigeminal nerves to reach many regions within CNS. About 40 substances have been suggested to reach the brain via the direct nose–brain

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pathway [19–22]. Therefore, targeting the brain via the nasal route provides potential for drug delivery, providing more opportunities for EM to enter the central nervous system and then act on central nervous system (CNS) disorders. However, whether this kind of direct delivery route exists or not for EM has not been investigated. In order to clarify this issue, EM formulations for nasal administration and intravenous injection were prepared; the direct nose–brain drug delivery of EM was investigated using brain microdialysis as sampling method. The EM concentration in plasma and CSF samples was determined by reversed phase-high pressure liquid chromatography (RP-HPLC) with fluorescence detection followed by data analysis and statistics.

2. Materials and methods

2.1. Materials

EM was purchased from Guangyi Pharmaceutical Factory, China. Polyvinylpyrrolidone (PVP) was got from BASF Corporation, USA. All other reagents were of analytical grade or the highest grade commercially available.

Microdialysis probes were U-shaped and made of hollow cellulose fiber (DM-22, 200 μm inner diameter and 220 μm outer diameter, EICOM CORP, Japan) and were used for both the in vitro and in vivo studies. The membrane was 4 mm in length with a molecular weight cut-off of 5000Da. Artificial CSF composed of 128 mM NaCl, 206 mM KCl, 1.26 mM $CaCl_2$ and 2 mM $MgCl_2$ was prepared using deionized distilled water. The solution was filtered through a 0.47 μm nylon filter before use.

2.2. Preparation of EM solution for nasal administration and intravenous injection

Solution 1 was prepared by dissolving PVP in water. Glycerol, EDTA-2Na and Benzalkonium Bromide were dispersed in water, which made solution 2. The two solutions above were mixed, water was added to 1000 ml. Then EM was added and stirred until dissolved. The final product was filtered through a 0.8 μm filter membrane and a 20 mg/ml EM solution for nasal administration was obtained.

A 5 mg/ml EM injection was prepared by simply dissolving EM with double distilled water with glycerol quantum satis added.

2.3. 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 12:12 h light–dark cycle) for at least 5 days before being used for experiments. Twelve Sprague–Dawley rats were randomly divided into two groups ($n = 6$/group). One group received intranasal drug treatment, the other group received intravenous treatment. The experimental procedures complied with the University Animal Ethics Committee Guidelines.

2.3.1. Probe implantation [25]

Throughout the experiment, the male Sprague–Dawley rats were anesthetized with an intraperitoneal injection of urethane (1.2 g kg^{-1}). The rats had their heads shaved and were placed in a stereotaxic apparatus. A midline incision of approximately 2 cm was made parallel to the sagittal suture. The bregma was located and used as the reference point for positioning the microdialysis probe. A microdialysis probe was stereotaxically inserted through a cranial burr hole made by a dental drill to a depth of 3.1 mm, using the following coordinates, in relation to bregma: 1.5 mm lateral, 0.9 mm posterior, and the probe was attached to the skull with dental cement.

2.3.2. Nasal cavity isolation and jugular vein cannulation

The rats were then held in a supine position with body temperature maintained at 37 °C under an infrared lamp. The nasal cavity was isolated from the respiratory and gastrointestinal tracts using a procedure described by Hirai [23] and Huang [24]. Briefly, after an incision was made in the neck, the trachea was cannulated with a polyethylene tube to maintain respiration. Another polyethylene-200 (PE-200) tube was inserted through the oesophagus toward the posterior part of the nasal cavity and ligated. The passage of the nasopalatine duct was sealed with an adhesive agent to prevent drainage of the solution from the nasal cavity to the mouth. A polyethylene tube was inserted into the jugular vein for intravenous injection and blood sampling.

2.3.3. Microdialysis procedure [25]

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. Before and during the implantation procedure the probe was rinsed with artificial CSF solution at a rate of 10 μl min^{-1}. Then, 5 min after implantation of the probe, the flow rate was reduced to 4 μl min^{-1} and was maintained at this level throughout the experiment. A recovery time of 1 h was allowed after the insertion of the sampling probe prior to drug administration.

2.3.4. Drug administration and collection of biological samples

For intranasal administration, 50 μl of the nasal EM formulation was instilled into the right nostril at a dose of 4 mg kg^{-1} via a microsyringe which was attached to a blunt needle. Blood samples (0.2 ml) were drawn from the tube in the jugular vein into heparin stabilized test-tubes at different times: 5, 10, 20, 30, 60, 90, 120, 150, 180, 240, 360 min post dosing. After each blood withdrawal, the same volume of sterile normal saline was put

back into the circulation to maintain total blood volume. CSF dialysate samples were collected at intervals of 20 min for 6 h. Plasma was separated by centrifugation at 8000 rpm for 15 min and kept frozen at −20 °C together with CSF dialysate for subsequent analysis. Intravenous administration was carried out by injecting 0.2 ml of the intravenous EM formulation at a dose of 4 mg kg^{-1} through an indwelling jugular vein cannula. The method of sample collection was the same as above. Drug administration in each group should be performed following the successful implantation of a microdialysis probe and stabilization for 1 h with artificial CSF.

2.4. Determination of the recovery of EM in cerebrospinal fluid (CSF)

The drug concentration in the CSF, collected by microdialysis, reflects the drug concentration in the extracellular fluid around the semipermeable membrane of the probe. However, as the dialysis procedure is not performed under equilibrium conditions, the concentration in the dialysate will be different from that in the periprobe fluid. The term recovery is used to describe this relationship.

2.4.1. In vitro recovery [25]

The zero-net flux method (ZNF) was applied to calculate the in vitro recovery. In this study, the microdialysis probe was immersed in artificial CSF containing EM (40.60 ng ml^{-1}) as a dialysis medium and perfused at 4 μl min^{-1} with artificial CSF solution containing different concentrations of EM (2.03, 10.15, 20.30, 40.60, 60.90, 81.20, 101.50 ng ml^{-1}, C_p). Microdialysate samples (C_d, 80 μl) were collected for each concentration ($n = 3$) of perfusion solution. The concentration difference between microdialysate samples and perfusion solution ($C_d - C_p$) was plotted against the concentration in the perfusion solution (C_p). The recovery was determined from the slope of the linear regression, while the abscissa intercept represented the concentration in the medium outside the probe [26].

2.4.2. In vivo recovery [25]

Retrodialysis method was performed to determine the in vivo recovery of EM. Three blank rats were prepared and microdialysis probes were implanted as described above and blank artificial CSF was perfused through the probes at 4 μl min^{-1} and stabilized for 1 h. After that, the perfusate was changed to three artificial CSF solutions containing EM 10.15, 40.6, 101.50 ng ml^{-1}, respectively, in turn and dialysates were collected every 20 min for 1 h. The in vivo recovery was calculated by the formula $R\,\% = (1 - C_d/C_p) \times 100\%$.

2.5. Analytical of EM in plasma and CSF

2.5.1. Pre-treatment of plasma samples

Briefly, 0.1 ml plasma was vortexed with acetonitrile (0.2 ml) for 5 min, and centrifuged for 5 min at 4000 rpm before the supernatant was transferred to a new glass tube, then 50 μl was injected onto the HPLC column. CSF dialysate samples were injected directly into the HPLC system for EM analysis without any pre-treatment.

2.5.2. RP-HPLC fluorescence analysis of EM

An HPLC method was developed and validated for EM analysis in rat plasma and CSF samples. The HPLC equipment (HITACHI, Japan) consisted of a HITACHI L-7110 Intelligent HPLC pump, and a HITACHI L-7200 Intelligent HPLC Autosampler, a HITACHI L-7420 Intelligent HPLC Detector, and a ANASTAR Chromatography Data System. Separation was achieved at 40 °C on a Kromasil C18 column (250 mm × 4.6 mm, particle size 5 μm, Zirchrom Company, Japan). The mobile phase consisted of acetonitrile–water–triethylamine (36:64:2 for both plasma and CSF samples), filtered and degassed under reduced pressure, prior to use. A guard column was used to prevent column clogging. Eluent was monitored by a fluorescence detector set at 290 nm (λex) and 348 nm (λem), and its flow rate was 1.0 ml min^{-1}.

2.6. Data analysis and statistics

Absolute concentrations in CSF were calculated from the concentrations in the dialysates using the following equation: $C = C_d/R$, where R is the in vivo recovery. The area under the concentration–time curve was calculated using the trapezoidal rule. To evaluate the brain-targeting efficiency, an index DTP% (direct transport percentage) was adopted and derived from Eqs. (1) and (2) as mentioned below [27].

$$\mathrm{DTP}\% = \{(B_{\mathrm{i.n.}} - B_x)/B_{\mathrm{i.n.}}\} * 100, \quad (1)$$

where

$$B_x = (B_{\mathrm{i.v.}}/P_{\mathrm{i.v.}}) * (P_{\mathrm{i.n.}}) \quad (2)$$

$B_x = \mathrm{AUC_{CSF}}$ fraction contributed by systemic circulation through the blood–brain barrier (BBB) following intranasal administration.
$B_{\mathrm{i.v.}} = \mathrm{AUC}_{0\to360}$ (CSF) following intravenous administration.
$P_{\mathrm{i.v.}} = \mathrm{AUC}_{0\to360}$ (plasma) following intravenous administration.
$B_{\mathrm{i.n.}} = \mathrm{AUC}_{0\to360}$(CSF) following intranasal administration.
$P_{\mathrm{i.n.}} = \mathrm{AUC}_{0\to360}$ (plasma) following intranasal administration.

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

3. Results

3.1. Analytical method

Due to the low EM levels in plasma or, of course, in CSF, almost all of the studies found in the literature have used radioimmunoassay (RIA) to determine the EM concentration in biological samples [28–30]. However, an RP-HPLC assay with fluorescence detection [31] was used in this study, due to the characteristic fluorescence absorption of EM. In plasma and CSF, EM can be detected sensitively without any interference, confirming that the analytical method used was suitable (Table 1).

3.2. In vitro recovery of microdialysis probe

The concentration difference between microdialysate samples and perfusion solution ($C_d - C_p$) was plotted against the concentration in the perfusion solution (C_p), shown in Fig. 1. The linear regression function was: $C_d - C_p = -0.4168C_p + 16.59$, $r = 0.9990$. The in vitro recovery was 41.68%, determined from the slope of the linear regression, while the abscissa intercept concentration (EM in the medium outside the probe) was 39.80 ng ml^{-1}, in agreement with the concentration of EM in the dialysis medium (40.60 ng ml^{-1}).

3.3. In vivo recovery

The results are shown in Table 2. Obviously, the recovery in vivo is lower than that in vitro. Using the recovery in vitro will lead to incorrect results.

Table 1
Formulation of EM for nasal administration

Formulation		Purpose for usage
EM	20.0 g	Active constituent
PVP	20.0 g	Thickening agent
glycerol	25 g	Isotonizing agent
Benzalkonium bromide	0.1 ml	Bacterial inhibitor
EDTA-2Na	0.5 g	Bacterial inhibitor
water	q.s., 1000.0 g	Solvent

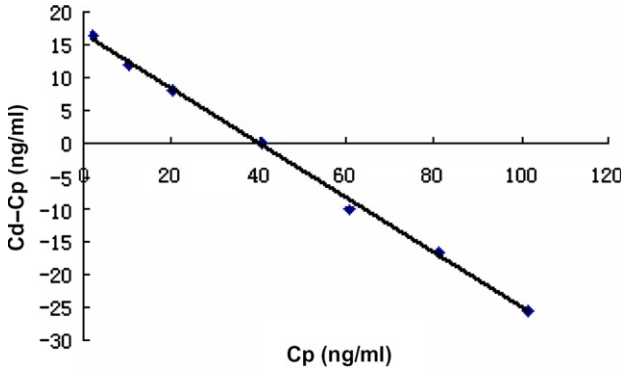

Fig. 1. The curve of $C_d - C_p$ and C_p ($n = 3$).

Table 2
In vivo recovery (%) of EM using retrodialysis ($n = 3$)

Rat number	EM concentration in perfusate (ng ml^{-1})		
	10.15	40.60	101.50
1	35 ± 4	36 ± 3	34 ± 9
2	38 ± 6	36 ± 2	36 ± 7
3	35 ± 3	35 ± 3	36 ± 8
Mean ± SD	36 ± 3	36 ± 2	35 ± 9

3.4. In vivo absorption studies

To determine whether or not EM is transported from the nasal cavity into the CSF, two groups of rats received EM intranasally or intravenously, respectively. EM reached a C_{max} (mean ± SD) at 107 min in plasma after intranasal administration (348.41 ± 19.47 ng ml^{-1}; Fig. 2), while in CSF, EM had a C_{max} of 87.35 ± 6.37 ng ml^{-1} (Fig. 3) at 20 min. For intravenous delivery, the C_{max} in plasma was 1255.51 ± 133.59 ng ml^{-1} (Fig. 2) but in CSF the value was delayed to 60 min (54.81 ± 4.92 ng ml^{-1}; Fig. 3). Table 3 summarizes the DTP (%) following intravenous and intranasal administration. The data after intranasal delivery differed significantly from those observed after intravenous injection ($P < 0.05$).

4. Discussion

In Fig. 1, the upper curve above the abscissa was the recovery by gain, while the lower part below the abscissa meant recovery by loss, representing the different direction that EM was transferred driven by the concentration gradient. However, their equivalency which was oblivious from the curve illustrated that the recovery rate was consistent and concentration independent, indicating little interaction of the compound to the membrane including non-specific binding. This guaranteed that the retrodialysis method we applied was reasonable to calculate the in vivo data.

It is generally accepted that small lipophilic compounds (MW < 500 Da) are absorbed across the nasal epithelium rapidly with a T_{max} ranging from 1 to 20 min post-dosing. The pharmacokinetics profile we got does not seem to comply with this (Fig. 3). Since in spite of its high lipophilicity ($\log P = 2.65$), EM has a mean molecular weight of about 680 Da and was transported to CSF in 20 min after nasal administration. It was reported that the molecular weight has more influence on the nasal absorption rate as compared to the lipophilicity [32]. However, the effect of molecular weight is also most likely to arise from the effective molecular size of the molecule [32], and the chemical structure of EM is more like cyclic rather than linear. Therefore, its quick absorption into CSF after nasal administration is understandable.

Reports in the literature reveal that the drug uptake into the brain from the nasal mucosa can occur via three different pathways [33,34]. One is the systemic pathway by which drug is absorbed into the systemic circulation

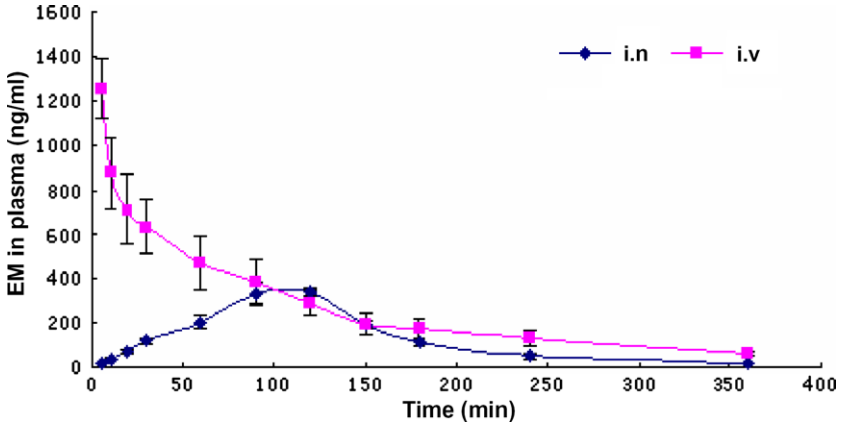

Fig. 2. Mean plasma concentration–time curves of EM in rats after i.n. and i.v. administration of EM at the dose of 4 mg/kg (mean ± SD).

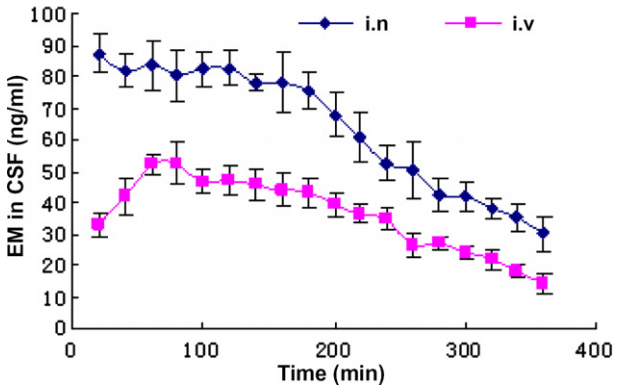

Fig. 3. Mean CSF concentration–time curves of EM in rats after i.n. and i.v. administration of EM at a dose of 4 mg/kg (mean ± SD).

Table 3
DTP (%) after i.n. and i.v. administration in rats (mean ± SD, $n = 6$) ($P < 0.05$)

	EM	
	i.n.	i.v.
B	21807 ± 1194	12491 ± 449
P	46071 ± 3811	92547 ± 18495
AUC_{CSF}/AUC_{plasma}	0.48 ± 0.05[a]	0.14 ± 0.04
Bioavailability (%)	53 ± 18	–
DTP (%)	70 ± 11	–

[a] Significantly different from that intravenous dosing, $P < 0.05$.

and subsequently reaches the brain by crossing the BBB. The others are the olfactory pathway and the trigeminal neural pathway by which partly the drug travels directly from the nasal cavity to CSF and brain tissue [34,35]. It can be concluded that the amount of drug that reaches the brain tissue after nasal administration is attributed to all of these three pathways. In order to evaluate the brain-targeting efficacy of the nasal formulation, the contribution of direct transfer to the brain is supposed to be separated from that of systemic distribution. Otherwise, as systemic brain distribution is rapid and extensive, direct transfer may be disguised and is therefore of less clinical importance. It is therefore important to use an appropriate pharmacokinetic study design. In this study, the CSF concentrations were determined by the microdialysis method with time intervals of 20 min for 6 h, enabling the calculation of AUC values instead of comparison of concentrations at various time points. This is actually the second study we have done to evaluate the direct nose–brain transport using microdialysis method. From other papers [36–38] and the results we got, microdialysis is a feasible way and might be easier compared to cannulating the cisterna magna or collecting the brain tissue to study the brain-targeting.

After nasal administration, the EM concentration reached a C_{max} value in CSF in 20 min and sustained for about 2 h as evident from the plateau-like curve (Fig. 3), which is remarkable because the nasal clearance half-life in rats is about 5 min [39]. This long time nasal absorption may be due to the use of PVP, which thickened the formulation and restricted the mechanical removal of mucociliary clearance and thereby prolonged the residence time of the administered solution in the nasal cavity [40]. While at the same time, EM concentration in plasma increased gradually and reached maximum after 107 min with its bioavailability about 53% (Fig. 2). This suggested that EM was not primarily reaching the CSF at 20 min by traveling from the nasal mucosa through the blood and crossing the BBB. The delayed absorption of EM across the nasal membrane into the systemic circulation might be resulted from the high lipophilicity ($\log P = 2.65$) of EM so that EM was accumulated in the mucosa and then slowly delivered into the blood.

Following intravenous administration, EM concentration in CSF should have reached a peak level rapidly as a result of the high initial plasma EM concentration (Fig. 2), and its high lipophilicity could also facilitate its passive diffusion across BBB. However, the T_{max} value in CSF was delayed to 60 min (Fig. 3). A similar phenomenon occurred in our previous study as well [25] ($\log P$ of estradiol is 4.01). One reason for this could be that the transport across the BBB was accompanied by the metabolism in blood which is more rapid than the one in CSF. The other reason could be the method of sampling the CSF, EM and

estradiol might be more likely to distribute to brain tissue rather than CSF due to their lipophilicity. Since the sampling method was parallel in both groups, it does not interfere with the result and conclusion.

Table 3 shows the great difference of AUC_{CSF}/AUC_{plasma} ratios between two groups (0.48 for nasal administration versus 0.14 for intravenous administration), which indicates a 3.4-fold increase in brain-targeting using intranasal delivery method. But this is not strong enough to judge whether there is a statistically significant difference or not because AUC_{CSF} contributed by systemic circulation through the BBB needs to be separated. It can be assumed that the AUC_{CSF} fraction contributed by systemic circulation through the BBB, divided by the AUC_{plasma} from the nasal route, is equal to that of the i.v. route (see Eqs. (1) and (2)). Therefore, DTP (%) represents the percentage of drug directly transported to the brain via the olfactory pathway and the trigeminal neural pathway. The remarkable values of DTP (%) indicate 70% of the drug administered intranasally reached the brain via direct pathways rather than by traveling through blood. Therefore, the direct nose–brain transport predominated. Besides, the low T_{max} value in CSF for intranasal delivery (20 min) compared to the one for intravenous delivery (60 min) also demonstrated the direct nose–brain transport of EM, because if EM was mainly absorbed from the nasal cavity into the systemic circulation and taken-up via the BBB into the brain, there will be a time delay in the distribution of EM into the brain.

Besides, benzalkonium bromide and EDTA-2Na applied in the present study are believed to be a mild bacterial inhibitor [41], guaranteeing that the nasal formulation is not aggressive.

The significant transport of EM to the CSF after nasal administration could be beneficial for the treatment of senile dementia and cerebrovascular insufficiency compared with the currently used oral administration. However, conclusions concerning the impact of the direct nasal transfer of EM in humans cannot be drawn from the results of rat studies because the olfactory area covers approximately 3% of the total nasal area in humans and 50% in rats [42]. The transport tendency can be consulted in future research. Besides, the potential for nasal transfer may increase with the development of new delivery devices and more effective formulations, improving the absorption and distribution. Studies clarifying the significance of nasal transfer of EM should therefore be included in future development of nasal administration of EM.

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