Distribution of nimodipine in brain following intranasal administration in rats[1]

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ABSTRACT

AIM: To determine whether nasally applied nimodipine (NM) could improve its systemic bioavailability and be transported directly from the nasal cavity to the brain. **METHODS:** NM was administered nasally, intravenously (iv), and orally to male Sprague-Dawley rats. At different times post dose, blood, cerebrospinal fluid (CSF), and brain tissue samples were collected, and the concentrations of NM in the samples were analyzed by HPLC. **RESULTS:** Oral systemic bioavailability of NM in rats was 1.17 %, nasal dosing improved bioavailibility to 67.4 %. Following intranasal administration, NM concentrations in olfactory bulb (OB) within 30 min post dose were found significant higher than in the other brain tissues. However, similar NM levels in different brain regions were observed after iv injection. AUC in CSF and OB from the nasal route was 1.26 and 1.39 fold compared with the iv route, respectively. The brain-to-plasma AUC ratios were significantly higher after nasal administration than after iv administration ($P<0.01$). **CONCLUSION:** Nasally administered NM could markedly improve the bioavailability and a fraction of the NM dose could be transported into brain via the olfactory pathway in rats.

INTRODUCTION

Over the last 20 years, intranasal drug administration has received considerable attention, because it is an attractive noninvasive route that can offer advantages such as rapid absorption, avoidance of liver first-pass metabolism, ease of convenience, and self-medication[1]. Recently it has also been reported a direct anatomical connection exists between the nasal cavity and the central nervous system (CNS). Various substances, including viruses, metals, dyes, peptides and some therapeutic agents have been shown to enter the brain via the olfactory pathway[2]. The nasal route, therefore, offers a potential for drugs targeting the brain.

Nimodipine (NM), isopropyl 2-methoxyethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate, is a dihydropyridine calcium-channel blocker and can be used in the treatment of cerebrovascular spasms and senile dementia. However, the clinical use of NM is severely restricted due to its extensive first-pass effect in liver. Oral bioavailability of NM ranges from 5 % to 10 % in human, which results in low amount in brain, the site of action. Therefore, the nasal route for the drug delivery to the brain appears to be an attractive alternative to oral administration.

The present study was undertaken to find out whether the nasal route could improve bioavailability of NM and NM could be directly transported from the nasal cavity to the brain after nasal delivery.

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MATERIALS AND METHODS

Drugs and reagents NM was supplied by Tianjin Central Pharmaceutical Factory (Tiangjin, China). NM was dissolved in ethanol-PEG400-water (4:3:3) mixture in a concentration of 1, 2, and 10 g/L for iv, oral, and nasal use, respectively. Nitrendipine was obtained from Shandong Xinhua Pharmaceutical Factory (Zibo, China) and used as an internal standard. Urethane was purchased from Shanghai Chemical Reagents Corporation. All other reagents were of analytical grade and commercially available.

Animal experiments Male Sprague-Dawley rats (252±35 g, Experimental Animal Center, Fudan University) were anesthetized with an injection of urethane (1.2 g/kg,ip) and kept on a heating pad to maintain the body temperature. Urethane had shown no inhibitory effect on neither retrograde nor anterograde axoplasmic transport, thus it appeared superior to other anesthetics for use in these studies[3]. The femoral artery was cannulated (PE-50) and attached to a syringe through a 3-way stopcock for blood collection. For intranasal administration, the trachea was cannulated with a polyethylene tube to aid breathing. About 20 min after the operation, 20-30 μL of NM solution was administered at a 2 mg/kg dose through each nostril, using a PE-10 tube attached to a microlitre syringe. Oesophagus was ligated immediately after dosing to reduce drainage of the drug. For iv administration, NM dosing solution (2 mg/kg) was injected through the femoral vein. The oral dose was 8 mg/kg and it was delivered to rats by gavage.

At 2, 5, 10, 15, 30, 60, 120, 240, and 360 min after administration of NM, blood samples of 500 μL were withdrawn from the femoral artery and centrifuged at 5000×*g* for 10 min, thus the plasma (200 μL) was separated. The blood collection was generally completed within 30 s. CSF samples were obtained by cisternal puncture from rats that received nasal and iv NM 2, 5, 10, 15, 30, 60, and 120 min after administration. Briefly, an incision was made in the skin over the occipital bone and the first layer of muscle was cut. After exposing the atlanto-occipital membrane, 80-100 μL CSF was taken through the membrane by inserting a 30-gauge needle, attached to a syringe. If blood appeared during sampling, the result was excluded. The animals were decapitated immediately after CSF sampling. The skull was cut open and the olfactory bulb (OB), olfactory tract (OT), cerebrum (CR), and cerebellum (CL) were carefully excised. The collected brain tissue specimens were washed in saline to get rid of blood-taint and blotted up with filter paper. Measurements were made on four rats at each time point. All samples, ie, plasma, brain tissues, and CSF were stored at -20 ºC until analysis.

Analytical procedures After thawing, the brain tissue samples were weighed and immediately homogenized with 1 volume of saline in a tissue homogenizer. NM in plasma and brain tissue were determined within 48 h of collection by HPLC after solvent extraction according to a modified HPLC method[4]. The plasma (200 μL) or brain homogenates (80-400 μL) was mixed with 50 μL of nitrendipine (2 μg/mL), 100 μL NaOH (1 mol/L) and 0.7 mL extraction solvent (n-hexane-diethylether, 1:1 v/v) were added to the test tubes. The mixture was vortexed for 2 min and centrifuged at 9000×*g* for 10 min. The organic phase was removed 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 ºC. The residue was reconstituted in 100 μL mobile phase and then 50 μL supernatant was injected onto an HPLC system (LC-10A, Shimadzu, Kyoto, Japan).

CSF samples were centrifuged at 9000×*g* for 20 min and 50 μL supernatant was analyzed by HPLC. Chromatographic separation was achieved at ambient temperature on a 4.6×200 mm C_{18} analytical column (Diamonsil™, Dikma) attached to a guard column (Nova-Pak, 10 μm, C_{18}, Waters). The mobile phase consisted of 0.05 mol/L ammonium acetate and acetonitrile in a ratio of 40:60 (v/v) for the plasma and brain tissue samples, and 35:65 (v/v) for CSF analysis. The flow rate was 1 mL/min. UV detection was set at 358 nm.

The linear range for NM in rat plasma, brain tissue and CSF samples were 15-1000 ng/mL, 15-4000 ng/mL and 10-150 ng/mL, respectively. The absolute analytical recovery from plasma and brain tissue for NM were 91 % ± 3 % and 83 % ± 3 % and the minimum detectable concentration were 10 μg/L plasma and 8 μg/L CSF, respectively.

Data analysis The initial iv concentration was obtained by fitting the plasma data of iv administration with 3p97 pharmacokinetic program. The area under the concentration-time curve (AUC) from 0 to the last data point was calculated by the trapezoidal rule. The variance for the AUC was estimated by the method of

Yuan[5]. The absolute oral or nasal bioavailability (F) of NM was calculated as the ratio of $AUC_{oral\ or\ nasal}$:AUC_{iv}, correcting for the differences in dose.

The brain-to-plasma AUC ratio was calculated to evaluate brain delivery of NM following intranasal administration. The variance for the AUC ratio was

$$\left(\frac{S_{\text{AUC ratio}}}{\text{AUC ratio}}\right)^2 = \left(\frac{S_{\overline{AUC_b}}}{\overline{AUC_b}}\right)^2 + \left(\frac{S_{\overline{AUC_p}}}{\overline{AUC_p}}\right)^2$$

approximated using the formula of propagation of error, where AUC_b and AUC_p represent the mean AUC value in brain tissue and plasma, respectively.

The statistics differences between nasal and iv treatment were assessed using Student's *t*-test.

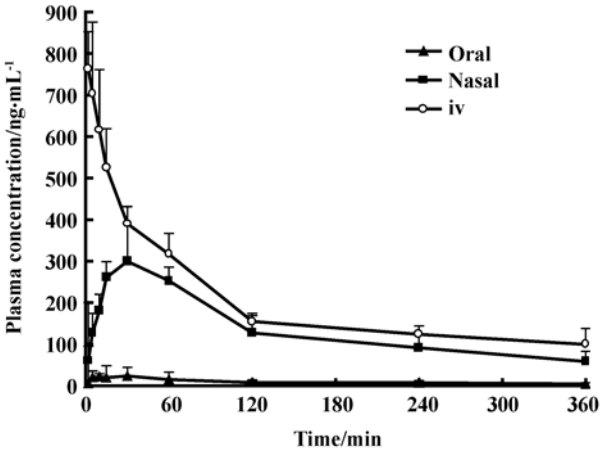

Fig 1. Plasma concentration of NM in rats after 8 mg/kg oral, 2 mg/kg nasal or 2 mg/kg iv administration. Mean±SD. *n*=4.

RESULTS

NM concentrations in plasma Plasma concentrations of NM following oral administration (8 mg/kg) were found near or below the limit of quantitation (15 μg/L) and oral bioavailability averaged 1.17 % of the dose (Tab 1). However, nasal absorption was rapid with the maximum concentration measured at 30 min (Fig 1). The nasal bioavailability of NM was 67.4 %.

NM concentrations in brain tissues and in CSF Following iv administration, brain NM concentrations reached peak levels at 2 min after dosing and declined exponentially as a function of time (Fig 2). Similar NM concentrations were found in different brain tissues. Following intranasal administration, the profile of NM levels in brain showed an initial absorption phase and maximum concentration achieved after about 5 min in OB and 15 min in remaining part of the brain. The highest concentration was observed in OB within 30 min post dose. The uptake of NM by brain tissues (OB, OT, CR, and CL) after oral administration was extremely low and the variances were large.

The concentration-time profiles in CSF (Fig 3) showed no increased concentration of NM after nasal compared to iv administration. However, a prolonged duration of the concentration was seen after nasal delivery.

Following nasal administration, the bioavailability was 125.7 % (2437±316 μg·min·L^{-1} *vs* 1939±132 μg·min·L^{-1}) in CSF and 138.9 % (103574±6922 ng·min·g^{-1} *vs* 74560 ±3617 ng·min·g^{-1}) in OB. The AUC in other brain tissues were slightly smaller than those

Tab 1. AUC of nimodipine after oral (8 mg/kg), nasal (2 mg/kg), or iv (2 mg/kg) administration in rats. Mean±SD. *n*=4-5.

Route	$AUC_{0\to360}$ (μg ·min·L^{-1} or ng ·min·g^{-1})					$AUC_{0\to120}$ (μg·min·L^{-1})
	Plasma	OB	OT	CR	CL	CSF
Oral	3368±512	2264±952	2178±641	2759±702	1363±419	-
Nasal	48302±3086	103574±6922	78831±6454	87647±8567	83327±6967	2437±316
iv	71655±2338	74560±3617	91588±4420	99391±4217	86564±3958	1939±132
F (oral)	1.17 %	0.76 %	0.59 %	0.69 %	0.39 %	-
F (nasal)	67.4 %	138.9 %	86.1 %	88.2 %	96.3 %	125.7 %

F: the absolute oral or nasal bioavailability of NM, correcting for the differences in dose. OB:olfactory bulb; OT: oflactory tract; CR: cerebrum; CL: cerebellum; CSF: cerebrospinal fluid.

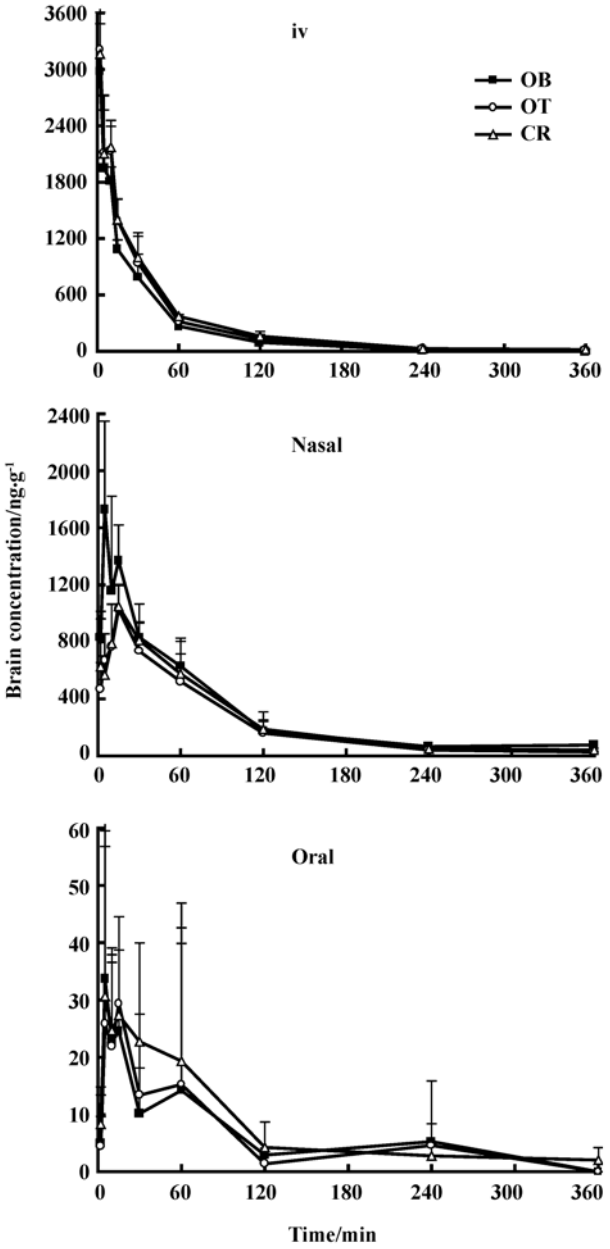

Fig 2. Brain NM concentration after 2 mg/kg iv, 2 mg/kg nasal, or 8 mg/kg oral doses. Mean±SD. *n*=4-5. OB: olfactory bulb; OT: olfactory tract; CR: cerebrum.

obtained after iv administration (Tab 1). Nevertheless, absolute bioavailability all less than 1 % in four brain tissues after oral dose was observed.

The ratios of AUC_{brain} to AUC_{plasma} after iv and nasal administration The ratios of AUC between brain to plasma at each time period in rats receiving nasal NM were significantly higher than in rats receiving iv injection (Tab 2). Up to 2 min after nasal delivery, the AUC ratio were about 7.4 times higher than that after iv administration (14±4 *vs* 1.89±0.19) in OB and about 16.4 times higher (0.21±0.08 *vs* 0.0128±0.0021) in CSF,

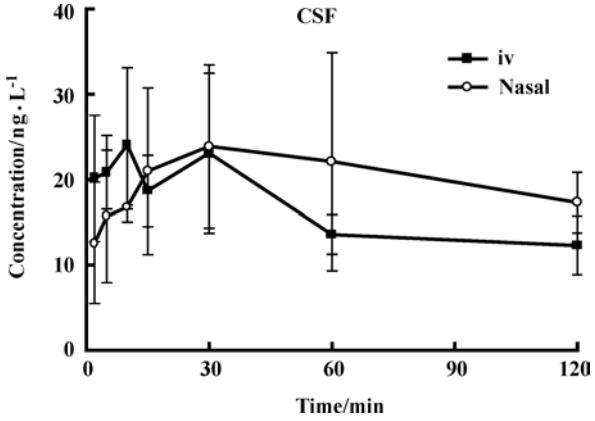

Fig 3. NM concentration in CSF following iv and nasal administration. Data represent the Mean±SD.

and up to 5 min post dose about 4.6 times higher (13±3 *vs* 2.8±0.3) in OB and about 7.3 times (0.16±0.04 *vs* 0.022±0.003) in CSF. These results suggested that when applied intranasally, a fraction of the NM dose could be transported directly from the nasal cavity to the brain.

DISCUSSION

NM is a highly potent calcium antagonist, but its oral bioavailability is relatively low due to high first-pass effect in liver. This appeared to be the case for NM in our study in rats. Although the dose of oral administration in rats was 4 times of that nasal dose, plasma levels following oral administration were extremely low, consequently lower amount of NM in brain was found. However, the absorption of NM from the nasal cavity into the systemic circulation was rapid and achieved relatively high bioavailability (67.4 %). Nasal delivery of NM, therefore, appears to be a viable alternative to oral administration.

The nose-brain pathway, as a conduit for transmission of agents into the CNS, is an area of ongoing research. It has been suggested that there is free communication between the nasal submusocal interstitial space and the olfactory perineuronal space, which appears to be continuous with a subarachnoid extension that surrounds the olfactory nerve as it penetrates the cribriform plate[6]. The direct anatomical connection affords some compounds the ability to access the CNS without the need to be transported from the systemic circulation across the BBB. In order to determine the existence of a direct nose-brain drug transport of nasally applied NM, in this research, a more complete pharmacokinetic examination of drug levels in plasma,

Tab 2. Brain-to-plasma NM AUC ratios as a function of time following iv and nasal administration. $^cP < 0.01$ *vs* iv administration. Mean±SD. *n*=4-5.

Time/ min	AUC_{brain}/AUC_{plasma}									
	iv administration					Nasal administration				
	OB	OT	CR	CL	CSF(×10^2)	OB	OT	CR	CL	CSF(×10^2)
0→2	1.9±0.19	2.0±0.15	2.0±0.18	1.9±0.14	1.3±0.21	14±4^c	7.8±2.8^c	10±4^c	8.1±2.8^c	21±8^c
0→5	2.8±0.3	3.0±0.3	2.9±0.3	2.8±0.24	2.2±0.3	13±3^c	6.2±1.2^c	7.1±1.7^c	6.2±1.2^c	16±4^c
0→10	2.8±0.3	3.1±0.3	3.1±0.3	2.9±0.3	2.7±0.3	9.8±2.3^c	5.0±0.7^c	5.2±0.7^c	5.1±0.7^c	12.3±18^c
0→15	2.7±0.23	3.1±0.3	3.1±0.3	2.9±0.21	3.0±0.3	7.7±1.4^c	4.6±0.5^c	4.7±0.5^c	4.7±0.5^c	10.4±11^c
0→30	2.4±0.14	2.9±0.17	2.9±0.17	2.6±0.15	3.7±0.3	5.2±0.7^c	3.6±0.4^c	3.8±0.5^c	3.9±0.4^c	8.8±12^c
0→60	2.1±0.13	2.5±0.15	2.5±0.15	2.2±0.13	4.2±0.4	3.8±0.5^c	2.9±0.4^c	3.1±0.4^c	3.0±0.4^c	8.6±13^c
0→120	1.6±0.10	1.9±0.11	2.1±0.11	1.8±0.10	4.7±0.4	3.1±0.3^c	2.4±0.3^c	2.6±0.3^c	2.5±0.24^c	9.4±13^c
0→240	1.3±0.07	1.5±0.09	1.7±0.09	1.5±0.08	-	2.4±0.23^c	1.9±0.21^c	2.1±0.3^c	2.0±0.22^c	-
0→360	1.0±0.06	1.3±0.08	1.4±0.07	1.2±0.07	-	2.1±0.20^c	1.6±0.17^c	1.8±0.21^c	1.7±0.18^c	-

OB: olfactory bulb; OT: olfactory tract; CR: cerebrum; CL: cerebellum; CSF: cerebrospinal fluid.

CSF and different brain tissues in rats has been used and the brain-to-plasma AUC ratio has been calculated to allow comparison of data collected following different routes of administration. The brain-to-plasma AUC ratios obtained after iv administration should represent the distribution of NM from systemic circulation into different brain tissues, if nasally applied NM enters CNS only via BBB, the brain-to-plasma AUC ratios after nasal dosing should be similar to those obtained after iv administration. However, in this study, the brain-to-plasma AUC ratios in different brain tissues and CSF from the nasal route were significant higher than those from the iv route and most pronounced in OB. Furthermore, NM concentration in samples collected within 30 min post dose was found to differ in different brain tissues following nasal application, the highest concentration was observed in OB. If the substance can be transported directly via the olfactory pathway, the first anatomical brain region of contact from the nasal cavity to the brain is the OB. Former studies have shown that the OB is a major passage in the nose-brain direct pathway[7], our results were in agreement with these studies and substantially supported the existence of a direct pathway.

Due to the high lipophilicity, NM might be more easily diffused from CSF further into brain tissues, its concentrations in CSF were low following intranasal and iv administration, which resulted in some analytical difficulties and high data variances. The concentration-time profiles in CSF after iv administration revealed a fast decline of the concentration between 10 min to 15 min and then increased till to 30 min (Fig 3). It was more likely due to animal individual variance (a rat only provided a CSF sample) and measurement error. CSF levels following oral administration of NM were found below the limit of detection (8 μg/L) in a previous experiment, consequently CSF did not be withdrawn after oral dose in this study.

NM is sparingly soluble in water. In order to investigate the brain targeting of NM after nasal administration, NM solution was prepared by using ethanol-PEG400-water (4:3:3) mixture. This formulation showed a certain irritation after nasal application in rats and it was not suitable for clinic use. For the effective exploitation of the nose to CNS delivery route for NM, an appropriate nasal delivery system warrants further investigation in the future.

It was concluded that nasally administered NM could avoid the first-pass metabolism in liver and markedly improve the bioavailability. Entry of a small fraction of the NM dose into brain via the direct nose-brain pathway after nasal delivery was confirmed.

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