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ORIGINAL ARTICLE

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Study on brain targeting of raltitrexed following intranasal administration in rats

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Abstract *Purpose*: To investigate the levels of raltitrexed (RTX) in blood and different brain tissues in rats and to find out whether there is any direct drug transport from nasal cavity to brain tissues following intranasal (i.n.) administration. *Methods*: Raltitrexed was administered to male Sprague-Dawley rats either intranasally or intravenously. Drug concentrations in blood and brain tissues were determined at different times post dosing. *Results*: The plasma levels achieved after i.n. administration were significantly lower than those following intravenous (i.v.) administration ($P < 0.05$) before 120 min; but were significantly higher ($P < 0.05$) after 120 min. Following i.n. administration, RTX concentrations in different brain tissues were constantly detected for quite a long time and differed significantly from each other, the rank order being $C_{OB} > C_{OT} > C_{CR} > C_{CL}$. On the contrary, RTX appeared only at the initial two or three time points in different brain regions after i.v. injection, and the concentrations were similar. AUC values in four brain regions by the nasal route were 54- to 121-fold compared with the i.v. route, the drug targeting index (DTI) values of nasal route were 71–158 for different brain regions, and about 99% of RTX content within 360 min in the brain were transported via the olfactory pathway. *Conclusions*: These results showed that antineoplastic RTX could be directly transported into the brain via the olfactory pathway in rats.

Keywords Raltitrexed · Intranasal administration · Blood–brain barrier · Brain targeting

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Introduction

It is well known that the blood–brain barrier (BBB) restricts the transport of most substances from the systemic circulation to the central nervous system (CNS) in order to maintain a stable environment. However, this barrier is also the main obstacle for the delivery of therapeutic substances to the brain, preventing many drugs from reaching there at therapeutic concentrations. As a result, treatment of some CNS diseases including CNS neoplasm by oral or intravenous (i.v.) administration still remains difficult. Therefore, brain-targeting delivery has been a challenging topic in brain diseases therapy in recent years.

In the past decade, the use of the nasal cavity as a route for drug delivery has been an area of great interest, especially for systemically acting drugs that are difficult to deliver via routes other than injection, 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-mediation [1]. Recently it has also been reported that a direct anatomical connection exists between the nasal cavity and the CNS that makes it possible to deliver some substances into the CNS by circumventing the BBB instead of penetrating it, which provides the basis for the development of therapeutic agents for intranasal (i.n.) administration. Many substances, including tracer materials, heavy metals, low molecular weight drugs and peptides have been shown to reach the CSF, the olfactory bulb (OB) and in some cases other parts of the brain, after nasal administration [2]. Drugs have been shown to reach the CNS from the nasal cavity by a direct transport across the olfactory region situated at the loft of the nasal cavity. It is the only site in the human body where the nervous system is in direct contact with the surrounding environment. The nasal route could be important for drugs that are used in crisis treatments, such as for pain, and for centrally acting drugs where the putative pathway from nose to brain

might provide a faster and more specific therapeutic effect [3]. The nasal route, therefore, offers a potential for drugs targeting the brain.

Raltitrexed (RTX), *N*-(5-[*N*-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-*N*-methylamino]-2-thenoyl)-L-glutamic acid (Fig. 1), which has a convenient dosing schedule of a single i.v. injection once every 3 weeks, is a specific, folate-based inhibitor of thymidylate synthase (TS), with activity in advanced colorectal cancer comparable with that of fluorouracil (5- fluorouracil) plus folinic acid. It is actively taken up into cells and then undergoes rapid, extensive metabolism to a series of polyglutamates, which results in potent TS inhibition, and also in prolonged intracellular retention of RTX [4–6]. One experiment demonstrated that the oral bioavailability of RTX in rats appeared to be low, which was approximately 10–20% [7], as a result, RTX could only be administered intravenously to patients in clinics. In another experiment, after i.v. administration to Rhesus monkeys at a high dose of 10 mg/m^2, the RTX penetrating into the cerebrospinal fluid (CSF) was estimated to range from 0.6% to 2.0% compared with that in plasma [8], which may account for why RTX had not been used to treat malignancies involving CNS. Wang et al. [9] demonstrated that methotrexate, which has both similar chemical structure with RTX (Fig.1) and some inhibitory activity against TS, could be directly transported from the nasal cavity into the CSF in rats. Another anti-cancer agent, 5-fluorouracil (5-FU), had also been delivered successfully to the brain of rats through the nasal route [10]. Therefore, the nasal route for delivering RTX to the brain appears to be an attractive alternative to i.v. administration, especially in CNS malignancy treating.

The present study was undertaken to investigate the concentration profiles of RTX in blood and different brain tissues after i.n. and i.v. administration in rats. Consequently, we could find out whether the nasal route could be used to transport RTX directly from the nasal cavity to the brain, bypassing the BBB. Also we could find the degree of drug targeting to the brain attained following RTX i.n. delivery.

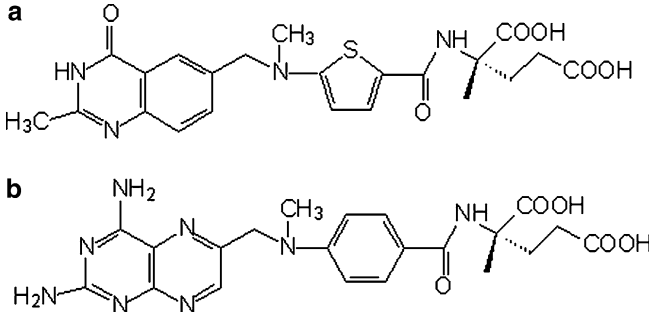

Fig. 1 Chemical structure of RTX and methotrexate. **a** RTX; **b** methotrexate

Materials and methods

Drugs and chemicals

RTX was supplied by Beijing Maijin Pharmaceutical and Technological Co. (Beijing, China), RTX was dissolved in phosphate buffer (0.1 M NaH_2PO_4, the pH was adjusted to 8.0 using 0.1 M NaOH solution) in a concentration of 1.00 and 10.0 mg/ml for i.v. and nasal use respectively. Sodium Pentobarbital was purchased from Beijing Chemical Reagents Company, methanol and acetonitrile were of HPLC grade, all other reagents were of analytical grade and commercially available.

Animal experiments

Male Sprague-Dawley rats weighing 230–270 g (Experimental Animal Center, AMMS, Beijing, China) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg) and kept on a heating pad to maintain normal body temperature. Throughout the whole experiment, rats were kept under anesthesia. The trachea and esophagus of the rat were surgically operated as previously reported [11] with some modification. Thus, an incision was made in the neck and the trachea was cannulated with a polyethylene tube (PE200) to allow free breathing. To prevent drainage of nasally dosed solution, the nasal cavity was isolated from the respiratory and gastrointestinal tracts by tying off the upper part of esophagus. For the i.n. administration, about 30 min after operation, 29–34 μl of the nasal formulation (10 mg/ml RTX in solution) was administered via a PE 10 tube attached to a microlitre syringe inserted 1 cm into each nostril of the rats at a dose of 2.5 mg/kg. For the i.v. administration, the RTX solution at a dose equivalent to 2.5 mg/kg was delivered through the tail vein and the injection volumes were between 0.58 ml and 0.68 ml. At 5, 15, 30, 60, 90,120, 240, 360 min for the i.n. administration and 2, 5, 15, 30, 60, 90,120, 240, 360 min for the i.v. administration after dosing, the animals were decapitated and blood was collected from the trunk. Then the skull was cut open and the OB, olfactory tract (OT), cerebrum (CR) and cerebellum (CL) 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 two volume of 0.1 M Tris (pH 10.0) by Ultraschallprozessor (UP 200S, Germany). Blood samples were anticoagulated with heparin and centrifuged at 3,000 rpm for 10 min to obtain plasma. All samples, i.e. aliquots of plasma and brain tissues homogenates, were stored for up to 48 h at −20°C until HPLC analysis. Measurements were made using four rats at each time point. The studies were approved by the local Ethic Committee for Animal Research.

Analytical procedures

RTX in plasma and brain tissue was assayed as monoglutamates according to a modified HPLC method [7]. To 100 µl of plasma samples or brain tissue homogenates, 200 µl methanol were added and vortexed for 2 min. After centrifugation at 3,000 rpm for 10 min, the supernatant was injected onto an HPLC system consisting of a LC-10AT VP solvent delivery system, SPD-10A *VP* UV-VIS spectrophotometric detector (Shimadzu, Japan), and N2000 chromatographic workstation (Intelligent information engineer Ltd. of Zhejiang University). Chromatographic separation was achieved at ambient temperature on a 4.6 mm×250 mm, C18 analytical column (Phenomenex, USA) attached to a guard column (Shim-pack GVP-ODS, 10 l×4.6, Shimadzu, Japan). The mobile phase was 25% methanol/15% acetonitrile/60% 0.175 M acetate acid (v/v) at a flow rate of 1 ml/min and a detection wavelength of 349 nm. The retention time was 8.0 min for RTX. Calibration curves of RTX were prepared with plasma and brain tissue spiked with known amounts of the drug utilizing its HPLC peak area ratio to RTX concentration by a weighted least square method. The linear ranges of RTX was 25.0–5,000 ng/ml and 75.0–15,000 ng/g and inter- and intra-day variations were less than 8.98%, 8.32% and 11.1%, 10.4% for plasma and brain tissue samples respectively. Plasma samples that had higher RTX concentrations above 5,000 ng/ml were diluted properly for determination. The extraction recoveries of RTX from plasma and tissue homogenates were in the range of 95.1% to 105.8%. The detection limits were 15.0 ng/ml and 45.0 ng/g for plasma and brain tissue samples.

Data analysis

Results obtained from the HPLC were plotted as drug concentration versus time curves in plasma or brain tissues. The C_{max} following i.v. injection was estimated by fitting the plasma concentration data to a conventional two-compartment model using a 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 ($AUC_{0 \rightarrow t}$) was calculated by the trapezoidal rule. The absolute nasal bioavailability of RTX was calculated as the ratio of $AUC_{i.n.}/AUC_{i.v.}$.

To evaluate the brain targeting after nasal dosing, drug targeting index (DTI) [9, 12] was described as the ratio of the value of $AUC_{brain\ tissue}/AUC_{plasma}$ following i.n. administration to that following i.v. injection. The higher the DTI is, the further degree of RTX targeting to the brain can be expected after i.n. administration.

$$DTI = \frac{(AUC_{brain\ tissue}/AUC_{plasma})_{i.n.}}{(AUC_{brain\ tissue}/AUC_{plasma})_{i.v.}}$$

To clarify nose–brain direct transport more clearly, a term 'brain drug direct transport percentage (DTP)' [13] was also introduced, which was defined based on Eqs. 1 and 2:

$$\frac{B_{i.v.}}{P_{i.v.}} = \frac{B_x}{P_{i.n.}} \quad (1)$$

$$DTP\% = \frac{B_{i.n.} - B_x}{B_{i.n.}} \times 100\% \quad (2)$$

where $P_{i.v.}$, $B_{i.v.}$, $P_{i.n.}$, $B_{i.n.}$, respectively, denote the $AUC_{0 \rightarrow 360}$ of RTX in plasma and brain tissue that obtained after i.v. and i.n. administration. B_x represents the brain AUC fraction contributed by systemic circulation through the BBB after nasal dosing. It is believed that drug uptakes into the brain from the nasal mucosa via two different pathways. One is the systemic pathway through which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing the BBB. The other is the olfactory pathway that the partial drug can travel from the olfactory region in the nasal cavity directly into CSF and brain tissue [2]. 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 RTX has been demonstrated in both rodents [7] and human beings [14–16], 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 the olfactory pathway.

The statistical differences between i.n. and i.v. administration were assessed using an unpaired student's *t*-test and a value of $P < 0.05$ was considered statistically significant. Results are presented as mean values ± SD.

Results

RTX concentration in plasma

Plasma concentrations of RTX versus time following i.v. or i.n. administration were shown in Table 1 and Fig. 2. At 2 min following i.v. injection, RTX attained a high concentration of 27,700 ± 3,240 ng/ml, and then declined quickly for 30 min, after which the decline became slow. Comparatively, after nasal administration, the peak plasma level was attained of 3,270 ± 765 ng/ml at 30 min, far lower than the maximal plasma concentration of i.v. injected RTX. During the period of 2–120 min, RTX concentrations in plasma after i.n. administration were all significantly ($P < 0.05$) lower than those after i.v. injection except the time points of 30 and 120 min. However, at 240 and 360 min, reversed results were observed, RTX in plasma after i.n. administration were all significantly ($P < 0.05$) higher than those after i.v. injection. Briefly,

Table 1 RTX concentrations in plasma following i.v. and i.n. administration at a dose of 2.5 mg/kg (Mean ± SD, $n=4$)

Time (min)	Plasma concentration (ng/ml)	
	i.v.	i.n.
2	27,700 ± 3,240	–
5	12,600 ± 4,970	523 ± 96
15	7,410 ± 2,100	838 ± 71
30	4,350 ± 1,510	3,270 ± 765
60	3,660 ± 721	2,280 ± 785
90	3,000 ± 136	1,780 ± 154
120	1,600 ± 51	2,040 ± 513
240	477 ± 94	1,300 ± 246
360	188 ± 55	948 ± 208

– time point not on schedule

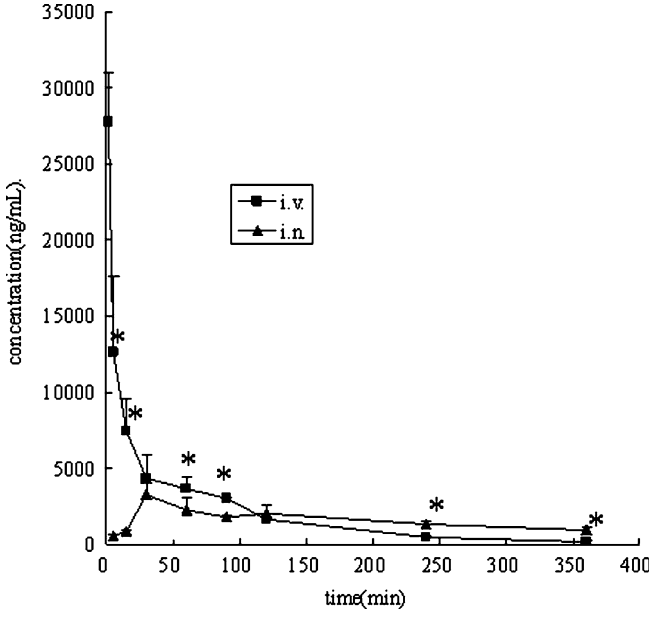

Fig. 2 Plasma concentrations of RTX in rats after i.v. or i.n. administration (dose: 2.5 mg/kg). (Mean ± SD, $n=4$). Any significant difference at each time point between two routes was calculated according to the Student's t-test: $^*P<0.05$

after i.n. administration of RTX, it performed a slower elimination than i.v. administration. The AUC of drug concentration curves in plasma were calculated, and the absolute bioavailability of RTX obtained following nasal administration was 76.6% (Table 2).

RTX concentration in brain tissues

As shown in Table 3 and Fig. 3, following i.v. administration, RTX concentrations in brain tissues could only be detected at the first two or three time points and the concentrations were very low. Furthermore, RTX concentrations in different brain tissues had no significant differences from each other. Following i.n. administration, RTX was delivered to the brain very quickly, and could be detected at 5 min in all brain tissues. The profile of RTX levels in the brain showed an initial absorption phase and a slow elimination phase. The maximum concentrations were achieved after about 30 min in OB and 60 min in the remaining part of the brain, and RTX concentrations even increased slowly at the end of the elimination phase in brain tissues following its nasal dosing. After nasal administration, the bioavailability was 9740% in OB, 12100% in OT, 9160% in CR and 5440% in CL, respectively (Table 2), and at each time point the RTX concentration in OB was much higher than that in other brain tissues (Table 3). The AUC values in four different brain tissues after i.n. administration were all much higher than those obtained after i.v. administration.

Drug targeting evaluation of nasal administration route

The calculated DTI and DTP values of OB, OT, CR and CL were shown in Table 4. Following i.n. administration, the DTI of RTX to OB, OT, CR and CL were 127,158,120 and 71 respectively, far greater than 1, which suggested there was a great portion of RTX targeting to the brain following i.n. administration. The results of DTP calculation further demonstrated that about 99% of RTX content within 360 min in the brain were transported via the olfactory pathway.

These data showed the existence of an alternative transport pathway to the brain other than the penetration across the BBB from the systemic circulation. RTX nasal administration might have the characteristic of brain targeting.

Discussion

RTX monotherapy is a conveniently administered alternative to 5-FU in the first-line treatment of ad-

Table 2 Pharmacokinetic parameters following i.v. and i.n. administration of RTX at a dose of 2.5 mg/kg. (Mean ± SD, $n=4$)

Parameters	Route	Plasma	OB	OT	CR	CL
C_{max} (ng/ml or ng/g)	i.n.	3,270 ± 765	1,870 ± 686	388 ± 324	201 ± 108	153 ± 57
	i.v.	27,700 ± 3,240	300 ± 176	153 ± 36	122 ± 19	166 ± 37
$AUC_{0\rightarrow 360}$ (ng min/ml or ng min/g)	i.n.	576,000	214,000	63,400	43,900	35,200
	i.v.	752,000	2,200	525	479	648
Ratio of $AUC_{i.n.}/AUC_{i.v.}$ (%)		76.6	9,740	12,100	9,160	5,430

i.n. intranasal, *i.v.* intravenous, *OB* olfactory bulb, *OT* olfactory tract, *CR* cerebrum, *CL* cerebellum

Table 3 RTX concentrations (ng/g) in brain tissues following i.v. and i.n. administration at a dose of 2.5 mg/kg (Mean ± SD, $n=4$)

Time (min)	i.v.				i.n.			
	OB	OT	CR	CL	OB	OT	CR	CL
2	300 ± 176	153 ± 36	122 ± 19	166 ± 37	–	–	–	–
5	128 ± 18	95 ± 15	116 ± 24	155 ± 19	1,870 ± 686	154 ± 61	86 ± 15	ND
15	124 ± 9	ND	ND	ND	145 ± 53	121 ± 41	ND	ND
30	ND	ND	ND	ND	1,480 ± 1,840	132 ± 26	110 ± 25	77
60	ND	ND	ND	ND	582 ± 286	388 ± 324	201 ± 108	153 ± 57
90	ND	ND	ND	ND	341 ± 208	106 ± 28	99 ± 16	109 ± 17
120	ND	ND	ND	ND	297 ± 156	138 ± 48	105 ± 9	91 ± 8
240	ND	ND	ND	ND	564 ± 192	182 ± 66	88 ± 16	96 ± 16
360	ND	ND	ND	ND	1,870 ± 686	180 ± 70	243 ± 90	131 ± 62

OB olfactory bulb, *OT* olfactory tract, *CR* cerebrum, *CL* cerebellum, – time point not on schedule, *ND* not detected

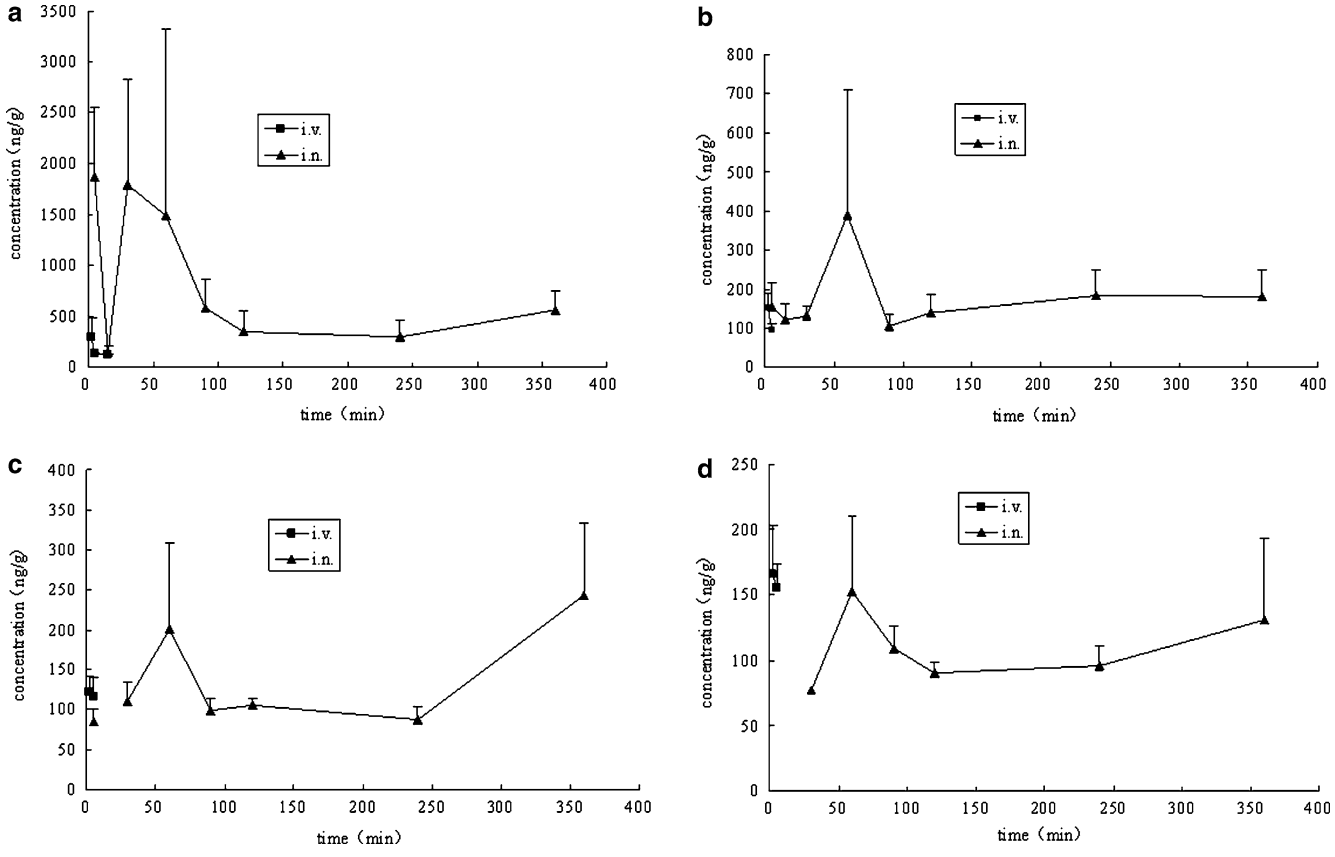

Fig. 3 Brain RTX concentrations after 2.5 mg/kg i.v. and nasal doses. (Mean ± SD, $n=4$). **a** olfactory bulb (OB); **b** olfactory tract (OT); **c** cerebrum (CR); **d** cerebellum (CL)

vanced colorectal cancer, and has single-agent activity in a variety of advanced solid tumors. While combined with other chemotherapeutic agents, it has demonstrated activity in patients with malignant mesothelioma, non-small-cell lung cancer, head and neck cancer, advanced gastric cancer and pancreatic cancer [17]. But under the situation when these cancer cells metastasize to the CNS, RTX will not act well because of its poor penetration across the BBB, unless by i.n. administration to circumvent the BBB. RTX acts on the same target enzyme (TS) with methotrexate and 5-FU, whichever had been delivered successfully from the nasal cavity to the CSF or the brain, intending to treat CNS neoplasm. Therefore, nasal administration of RTX is a promising way

Table 4 Drug targeting index and DTP values of RTX in different brain tissues following i.n. administration at a dose of 2.5 mg/kg

Brain tissues	DTI	DTP (%)
OB	127	99.2
OT	158	99.4
CR	120	99.2
CL	71	98.6

OB olfactory bulb, *OT* olfactory tract, *CR* cerebrum, *CL* cerebellum

for CNS neoplasm treating especially in meningeal spread of solid tumors, which remains a difficult therapeutic problem in the clinic.

The BBB denies many therapeutic agents access to brain tumors and other diseases of the CNS, even more than 98% of drugs of smaller molecular size do not cross the BBB [18]. There are many methods potentially to overcome the obstacle of BBB—the nose–brain pathway, as a conduit for transport 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 [19].

It has been proved convictively that not only small molecular weight substances, but also large ones can circumvent the BBB by the nose–brain pathway. Intranasally administered proteins or peptides, e.g. wheat germ agglutinin-horseradish peroxidase [20], nerve growth factor (NGF) [21, 22], insulin [23–25], and vasoactive intestinal peptide (VIP) [26, 27] were demonstrated to reach the CNS. Dufes et al. [27] demonstrated that intranasally administered VIP (at least in part) reached the brain as intact molecules, but after i.v. infusion no intact VIP was found in the brain. VIP is an interesting model to evaluate the potential advantages of the i.n. over the i.v. route for brain delivery of peptides. All of these results hinted at a direct pathway for large molecular weight substances from the nose to the brain.

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, there is little information about the direct nose–brain transport of nasally applied RTX, a new drug approved in 1996 in Britain. The oral bioavailability of RTX reported was low in rats [7], but in our study, the absorption of RTX from the nasal cavity into the systemic circulation was rapid and achieved relatively high bioavailability (76.61%). Nasal delivery of RTX, therefore, appears to be a feasible alternative to oral administration.

Some studies showed that lipophilic drugs, which can easily go across the BBB, did not show any expected increased uptake in brain tissues after nasal administration. Dahlin et al. [28] have studied the nasal administration of (S)-UH-301, a relatively lipophilic drug (log $P = 4.03$). The concentration–time profiles in CSF showed no increased concentration of (S)-UH-301 after nasal administration, compared to i.v. administration. Hussain et al. [29] considered that lipophilic drugs could be rapidly cleared to the systemic circulation from the nasal cavity, therefore, the transport via the nasal epithelium into CNS became insignificant. On the contrary, hydrophilic drugs, which can hardly penetrate the BBB, could be easily delivered from the nasal mucosa to the brain. Wang et al. [9] reported that methotrexate (log $P = -1.85$) was preferential to enter CSF after nasal administration as compared to i.v. administration in rats. The AUC_{CSF} ratio of i.n. dosing was more than 13 times as high as i.v. injection. This is also the case with RTX, whose log P was calculated to be −0.92 (at http://www.logP.com). In our study, the ratios of $AUC_{i.n.}/AUC_{i.v.}$ in different brain regions ranged from 54.35 to 120.85. Therefore we can conclude that, for drugs with poor BBB penetration, it may be hopeful to obtain high brain drug concentrations while administering nasally.

In order to more clearly understand nose–brain direct transport following RTX's i.n. administration, we introduced two terms, one is 'DTI', and another is 'DTP'. The calculated values of DTI and DTP strongly support the existence of an alternative brain entry pathway for RTX from the nasal cavity, and with the help of DTP, we could easily distinguish the RTX transported directly from the nasal cavity to the brain from the apparent distribution of RTX in the brain after i.n. administration.

In our study, RTX content differed considerably in different brain regions after i.n. administration compared with that after i.v. administration. The highest concentration was observed in the OB, followed by the OT, then CR and CL. These findings are in good agreement with the results reported by Chow et al. [30] and Zhang et al. [31] for the i.n. administration of cocaine and nimodipine respectively. RTX's concentrations measured in the OB and tract are much greater after i.n. administration than in the CR and CL, which obviously indicated that RTX diffused from the nasal cavity to the OB, OT, CR and CL successively and there existed a nose–brain direct pathway. The great difference may limit the utility of the i.n. approach to some extent, but the RTX concentrations in different brain regions were measured only for 6 h in our study, as time goes on, the difference will become progressively less. There was marked variation in the drug concentration measured after i.n. administration in rats over time, which could be explained by its significant inter-animal variation, and was consistent with its pharmacokinetic parameters of patients reported early.

In our experiments, RTX showed a prolonged duration in both plasma and brain tissues after nasal administration, inconsistent with the report of (S)-UH-301 [28], which only kept longer in plasma. This may be owing to RTX's hydrophilicity, which resulted in its accumulation in the nasal cavity and continuous transport into the systemic circulation via the whole nasal mucosa or into the brain tissues via the olfactory mucosa respectively. However, at the former two or three time points after i.v. administration, very high concentrations of RTX in plasma and very low concentrations of it in brain tissues were detected, which may be explained by the passive diffusion of RTX across the BBB was difficult to a certain extent. Moreover, we also found that RTX concentrations even increased slowly at the end of the elimination phase in brain tissues following its nasal dosing. This phenomenon may be explained not only by its accumulation in the nasal cavity and con-

tinuous transport into the brain tissues via the olfactory mucosa, but also by its different elimination speed between brain tissues and plasma.

In brain targeting studies of nasal administration, some researchers would like to determine drug concentration in the CSF only [9, 28, 32], others would like to do so in both CSF and brain tissues [13, 31, 33, 34]. But in fact, drug concentration of CSF may not be consistent with that of brain tissues precisely, Zhang et al. [13, 31] reported that the drug level in CSF was much lower than that in different brain tissues following i.n. administration. Therefore, in our study, we preferred to determine RTX in the different brain regions which are the pharmacologically relevant targets in CNS diseases treatment.

Rats were the most-frequently used animal models in the nasal route for brain targeting researches, the supine or prone position of the rat in nasal absorption experiments has a large influence on the drug absorption across the nasal mucosa and consequent distribution in blood, CSF or brain tissues. Generally, the supine position can assure drugs' full contact with a larger area of nasal mucosa and have a better absorption into systemic circulation than the prone position. In our experiments, all rats took the supine position in both i.v. and i.n. administration, which might explain RTX's relatively high bioavailability following i.n. administration.

It would be necessary to consider the anatomical differences between rats and human beings—the olfactory and respiratory epithelia of the rat are interspersed throughout the entire nasal mucosa, while in humans the olfactory epithelium is present only at the roof of the nasal cavity. It seems disadvantageous for the drug transport into CNS via the olfactory pathway when applied in human compared with rodents. To overcome this disadvantage, the retention time of the drug over the olfactory areas should be prolonged by using viscous solutions or gels for nasal dosing; we can also use certain devices (e.g. insert a soft tube into the human nasal cavity to the olfactory areas) to apply the drug to the olfactory areas or let patients keep lying on their backs after i.n. administration, which are more feasible solutions especially in RTX's dosing, a single dose every 3 weeks.

Intranasal administration can be useful only if it does not promote either histological or functional toxicities. In the case of RTX, we can predict that its nasal delivery will not cause severe histological or functional toxicities because its administration interval is more than 20 days. The related studies are being undertaken now. Another problem to be considered is the potential for CNS toxicity of RTX after i.n. administration, although RTX is administered on a q 3 week schedule, its potential for CNS toxicity should be evaluated in the future. Since brain targeting following i.n. administration is a just progressing area, there is nearly no report of CNS toxicity of any therapeutic agents after i.n. administration. However, the toxic study of CNS is very important and its importance will be recognized by more and more researchers.

In conclusion, nasal administration of RTX could avoid the first-pass metabolism and markedly improve the bioavailability. It was confirmed that the antineoplastic RTX can be directly transported from the nasal cavity to the brain and there was a large fraction of the RTX dose into the brain via the direct nose–brain pathway after nasal delivery in rats. The RTX concentrations in brain tissues after i.n. administration kept steady in a certain range and lasted for a quite long time. It may be helpful for both increasing the brain therapeutic levels and reducing the systemic side effects following nasal administration of RTX. So RTX's nasal administration promises to become an effective noninvasive route for certain brain cancers' prophylaxis and treatment, although it has not been confirmed clinically.

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