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ORIGINAL ARTICLE

Effect of polysorbate 80 on the intranasal absorption and brain distribution of tetramethylpyrazine phosphate in rats

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Abstract

Drug delivery to the brain is limited by the blood-brain barrier (BBB). Intranasal delivery is a non-invasive route of drug administration which can bypass the BBB and contributed to a direct and rapid transport of drugs to the brain. However, intrinsic drug distribution to the brain after intranasal administration may not be sufficient to achieve required clinical efficacy. In this study, taking 2,3,5,6-tetramethylpyrazine (TMPP) as a model drug, the feasibility of using polysorbate 80 as an absorption enhancer and message guider to increase drug distribution in the brain was employed. After intravenous/intranasal administration of TMPP formulations with/without polysorbate 80, drug concentration in both plasma and brain was measured at specific time points, and the pharmacokinetic parameters were compared. It was demonstrated that compared with intravenous administration, brain targeting efficiency of TMPP was improved remarkably by intranasal route. Upon intranasal administration, the addition of polysorbate 80 significantly increased TMPP concentration in both plasma and brain linearly up to polysorbate 80 concentration 2%. Based on drug targeting efficiency, drug targeting index, and nose-to-brain direct transport percentage, polysorbate 80 decreased the nose-to-brain direct transport ratio of TMPP in a polysorbate 80 concentration-dependent manner although the total brain targeting efficiency was unchanged, with significantly enhanced absolute drug concentration in the brain achieved. In summary, polysorbate 80 is a promising excipient to increase drug concentration in both plasma and brain via intranasal route.

Keywords Polysorbate 80 · Absorption · Brain distribution · Nasal · Tetramethylpyrazine phosphate

Introduction

The blood-brain barrier (BBB), characterized by restrictive continuous endothelium [1], is the main obstacle preventing drugs from permeating into the brain and limits the therapy of most brain-related diseases. To overcome the BBB and deliver drugs to the central nervous system, many strategies have been taken, which can generally be divided into five categories, including direct injection and implantation, temporary disruption of the BBB, drug manipulations, nanostructure system, and nasal delivery [2]. Among them, intranasal drug delivery has attracted extensive attention because it is a non-invasive route of drug administration, which can bypass the BBB and lead to a direct and rapid drug delivery to the brain [3]. After intranasal administration, drugs can be transported to the brain mainly by four pathways, namely olfactory nerve pathway, trigeminal nerve pathway, vascular pathway, and the lymphatic pathway [4]. Besides, the nasal cavity featured with rich vascularization, porous and thin endothelial basement membrane, and fairly wide absorption area [5] is an alternative administration route for systemic absorption. It can be used to avoid the first-pass metabolism of some small molecular drugs [6] to improve their bioactivity.

However, intrinsic drug distribution to the brain after intranasal administration may not be sufficient to achieve required clinical efficacy. Therefore, many other strategies are attempted to further increase drug concentration in the brain from different aspects. Ligand-mediated therapy is one of the strategies to enhance drug penetration through the BBB by triggering the receptor-mediated transport [7]. However, low brain permeability of the antibodies and high cost limited the potential application of antibody-mediated transport through BBB [8]. Polysorbate 80, a kind of non-ionic surfactant, has been extensively reported to be able to enhance drug transport to the brain by coating on the surface of nanoparticles via different administration routes. For

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example, for intravenous administration, a significant increase of drug concentration in the brain was observed for 1% polysorbate 80-coated poly(n-butylcyanoacrylate) nanoparticles compared to the uncoated nanoparticles and the free drug [9–11]. And it was found that chitosan nanoparticles coated by polysorbate 80 specially deposited in the frontal cortex and cerebellum after intravenous injection [12]. Besides, nanoparticles coated with 1% polysorbate 80 realized longer circulation in the blood circulation by decreasing the accumulation of drug tacrine in the liver and spleen after intravenous administration [10]. Subsequently, intranasal delivery of 1% polysorbate 80-coated neurotoxin-loaded nanoparticles promoted higher drug distribution in the brain compared with the free drug solution and intravenous injection of drug-loaded nanoparticles [13, 14]. Moreover, the bioavailability and brain distribution of quercetin were enhanced after oral administration of polysorbate 80-coated poly(n-butylcyanoacrylate) nanoparticles [15]. Apart from the physical absorption on the nanoparticles, it was also found that poly(propyleneimine) dendritic nanoconjugate chemically modified with polysorbate 80 has the potential to deliver significantly higher amount of drug to the brain tumor with improved therapeutic outcome after intravenous injection [16]. The enhanced brain distribution after intravenous injection of polysorbate 80-coated nanoparticles is explained as that nanoparticles absorb apolipoproteins from the blood and then mimic lipoprotein particles to be taken up by brain capillary cells via receptor-mediated endocytosis [17]. However, the fact that polysorbate 80-coated nanoparticles can enhance drug distribution in the brain not only after intravenous injection but also after oral and intranasal administration reminds us that polysorbate 80 might function as a "message drug" to increase drug distribution in the brain. Moreover, polysorbate 80 is a well-known absorption enhancer in pharmaceutical field; the desorption of coated polysorbate 80 from the nanoparticle surface might also exert an absorption enhancing effect.

Therefore, using 2,3,5,6-tetramethylpyrazine (TMPP), a biologically active ingredient originally isolated from *Ligusticum wallichii* France and widely used for the treatment of cardiovascular and cerebrovascular diseases in China [18], as a model drug, the feasibility of using polysorbate 80 alone in solution to enhance drug absorption in the brain after intranasal administration was explored. Moreover, the influence of polysorbate 80 concentration on brain targeting efficiency and nose-to-brain direct transport percentage of TMPP was elucidated.

Materials and methods

Materials

TMPP was a gift from Beijing Shuanghe Inc. Polysorbate 80 (Tween®80) was purchased from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). Carbamazepine (99.6% purity, internal standard) was obtained from Shandong Xinhua Pharmaceutical Company, Ltd. (Jinan, China). Urethane was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shenyang, China). Methanol of liquid chromatographic grade was purchased from Tianjin Concord Tech Reagent Company (Tianjin, China). All other chemicals were of analytical grade.

Preparation of TMPP formulations

For intranasal drug delivery, TMPP was dissolved in 7% nicotinamide solution to reach a concentration of 20 mg/mL. Thereafter, appropriate amounts of polysorbate 80 (0%, 0.5%, 1%, 2%, and 5% (*w*/*v*)) were added to the above solution. pH of the solution was adjusted to 6 using 0.1 M sodium hydroxide. Isotonicity was adjusted by adding appropriate amount of sodium chloride.

Intravenous formulation was prepared by dissolving TMPP (4 mg/mL) in the saline solution and adjusting pH of the solution to 7.

In vivo analytical method of TMPP

TMPP levels in blood plasma and brain were determined by HPLC. The concentration of TMPP in the blood plasma was measured according to the method described previously [19].

TMPP concentration in the brain was measured according to the following method. Briefly, the collected brain tissue was added to saline solution of the same weight. Then, the solid brain was homogenized in a homogenizer. One hundred microliters of the homogenized solution was added to 5 μL 50 μg/mL carbamazepine solution (internal standard) and then vortexed after adding 150 μL methanol for protein coagulation. Thereafter, the sample was centrifuged and the supernatant was used for HPLC analysis as described previously [19]. The limit of detection and quantitation of TMPP was 0.08 ng and 0.2 ng, respectively. There was a linear correlation between As/Ai and TMPP concentration in the range of 0.01–10 μg/mL (As/Ai = 0.2711c + 0.0506, $n = 7$), where As is the peak area of TMPP and Ai is the peak area of the internal standard. The method and extraction recoveries of TMPP from brain tissue at three different concentrations were 100.00 ± 5.89%, 102 ± 2.8%, and 106 ± 4.01% and 87.88 ± 3.47%, 81.95 ± 4.17%, and 84.87 ± 4.86%, respectively. The inter-day and intra-day relative standard deviations (RSDs) were less than 5.88% and 6.25%, respectively.

In vivo study in rats

The animal experiment was carried out in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985). Male Wistar rats (7 weeks old, 200 ± 20 g) were supplied by the Lab Animal Center of Shenyang Pharmaceutical University. The experimental protocol was

approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals.

Experiments were performed in 180 healthy male Wistar rats weighing between 180 and 220 g. The animals had access to food and drink ad libitum and were divided into six groups before experiment. One group is for intravenous administration as a control. Other groups were named as 0% polysorbate 80 group (control group), 0.5% polysorbate 80 group, 1% polysorbate 80 group, 2% polysorbate 80 group, and 5% polysorbate 80 group for intranasal administration. The rats were anesthetized by intraperitoneally injecting urethane at a dose of 1.0 g/kg. The nasal formulations were administered using a microsyringe (Hamilton Bonaduz AG, Switzerland) attached via a needle to a short polyethylene tube inserted approximately 0.7 cm into the nostril. For intravenous administration, a bolus injection was administered via the tail vein. For all the groups tested, the formulations were administered at a dose of 3.2 mg TMPP/kg. At 1, 5, 30, 60, 90, and 120 min post administration, the rats (5 animals per time point) were sacrificed by decapitation to withdraw the blood (about 0.5 mL). All the blood samples were collected in heparinized tubes and immediately centrifuged at 4000 rpm for 15 min. The precipitate was separated to obtain the plasma sample. And the brains were removed and weighted. The plasma and brain samples were stored at − 20 °C until analysis.

Pharmacokinetic and brain targeting evaluation

The peak plasma concentration (C_{max}) and the time to reach the peak concentration (T_{max}) were determined directly from the plasma concentration-time curves. Drug brain targeting efficiency (DTE) of the drug was calculated by using Eq. (1), which represents a time-average partitioning ratio [20, 21].

$$\mathrm{DTE} = \mathrm{AUC}_{\mathrm{brain}}/\mathrm{AUC}_{\mathrm{plasma}} \quad (1)$$

Drug targeting index (DTI) and nose-to-brain direct transport percentage (DTP) was calculated by Eqs. (2) and (3), respectively.

$$\mathrm{DTI} = \frac{\left(\mathrm{AUC}_{\mathrm{brain}}/\mathrm{AUC}_{\mathrm{plasma}}\right)_{\mathrm{i.n.}}}{\left(\mathrm{AUC}_{\mathrm{brain}}/\mathrm{AUC}_{\mathrm{plasma}}\right)_{\mathrm{i.v.}}} \times 100\% \quad (2)$$

$$\mathrm{DTP} = \left(\frac{B_{\mathrm{i.n.}} - B_x}{B_{\mathrm{i.n.}}}\right) \times 100\%, B_x = \left(\frac{B_{\mathrm{i.v.}}}{P_{\mathrm{i.v.}}}\right) \times P_{\mathrm{i.n.}} \quad (3)$$

where $P_{i.v.}$, $B_{i.v.}$, $P_{i.n.}$, and $B_{i.n.}$ denote the $AUC_{0\rightarrow 120}$ of TMPP in plasma and brain tissue after intravenous and intranasal administration, respectively. B_x represents the fraction of brain AUC, which is contributed by redistribution of the systemically absorbed drug via nasal mucosal to the brain through BBB.

DTI is used to evaluate the degree of drug targeting to the brain after intranasal administration. The higher the DTI is, the better drug targeting to cerebrospinal fluid can be expected after intranasal administration [22]. DTP represents the percentage of drugs directly transported from the nose to the brain via other pathways such as olfactory pathway [3].

Statistical analysis

The pharmacokinetic parameters were obtained using DAS 2.0. Data are presented as means ± standard deviations from five experiments. The statistical significance was determined using one-way analysis of variance (ANOVA) followed by the Dunnett test. Probability values $p < 0.05$ were considered significant.

Results

In vivo absorption of TMPP after intranasal delivery

Previous study demonstrated that pH 6.0 is the optimal pH for the intranasal absorption of TMPP [19]; therefore, all the formulation investigated in this study was fixed at pH 6.0. Based on the physiological structure of the nose and its thin mucosa, it is assumed that nasal drug delivery could inherently enhance drug distribution in the brain; therefore, first of all, drug plasma concentration was measured and compared after intranasal administration and intravenous injection. Their pharmacokinetic parameters are summarized in Table 1. As shown in Fig. 1a, after intranasal administration, the T_{max} of TMPP in the plasma was around 5 min, indicating fast absorption of TMPP in the nasal cavity, which is in good agreement with the rapid onset of intranasal drug delivery system. Meanwhile, it was noted that

Table 1 Comparison of the pharmacokinetic parameters of TMPP after i.n. and i.v. administration of the formulations without polysorbate 80 in rats (means ± SDs, $n = 5$)

Parameters	i.v.	i.n. control group
$(AUC_{0-120})_{plasma}$ (μg min/mL)	774.5 ± 34.6	322.8 ± 14.2*
$(AUC_{0-120})_{brain}$ (μg min/mL)	41.6 ± 1.33	85.4 ± 3.6*
$(C_{max})_{plasma}$ (μg/mL)	48.9 ± 0.6	16.7 ± 2.26*
$(C_{max})_{brain}$ (μg/mL)	0.8 ± 0.1	3.5 ± 0.4*
$(T_{max})_{plasma}$ (min)	1 ± 0	5 ± 0*
$(T_{max})_{brain}$ (min)	1 ± 0	5 ± 0*
$(MRT_{0-120})_{plasma}$ (min)	21.9 ± 1.5	15.6 ± 0.2*
DTE	0.054 ± 0.001	0.265 ± 0.02*
DTI (%)	–	490.7 ± 37.0
DTP (%)	–	79.68 ± 0.89

*$p < 0.05$, compared to the i.v. administration

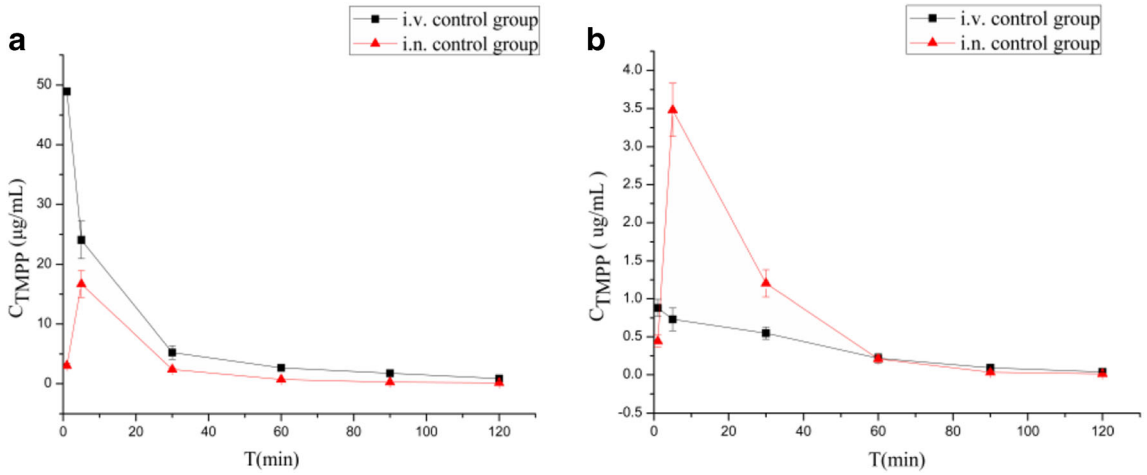

Fig. 1 Mean TMPP concentration-time profiles in plasma (**a**) and brain (**b**) following intravenous (i.v.) and intranasal (i.n.) administration without polysorbate 80 in rats (means ± SDs, $n = 5$)

the C_{max} was much lower compared to that of intravenous injection, implying the potential to decrease systemic side effect after intranasal drug delivery. The absolute bioavailability of TMPP after intranasal administration was 41.68%. Compared to oral drug administration, which has the absolute bioavailability about 10–30% [23], intranasal drug delivery improved the bioavailability of TMPP significantly.

To elucidate the influence of administration route on brain distribution, drug concentration in the brain after intravenous and intranasal administration was measured and presented in Fig. 1b. After intravenous injection, TMPP concentration in the brain was measurable 1 min after administration, indicating TMPP could penetrate the BBB to be distributed in the brain after systemic circulation, although drug concentration in the brain was quite low (< 1 μg/mL). In contrast, drug concentration in the brain was significantly increased after intranasal administration, especially in the first 60 min, with C_{max} approximately 3.5-fold higher than that of intravenous injection, and twofold increase in $(AUC_{0–120})_{brain}$ was also noted after intranasal drug administration, indicating dramatically improved drug distribution in the brain via intranasal route.

Drug brain targeting efficiency was evaluated by comparing DTE value, 0.054 and 0.265 for intravenous injection and intranasal administration, respectively, implying that brain targeting can be improved by 490.7% via intranasal route.

In addition to vascular pathway, drugs can also be distributed to the brain via other routes [24]. Therefore, in order to clarify what is the percentage of drug transported directly from nose to the brain, drug direct transport percentage (DTP) was calculated; it was 79.68%, indicating that 79.68% of the intranasally administrated drug was transported to the brain by other pathways such as olfactory pathway, trigeminal nerve pathway, or lymphatic pathway [4].

Effect of polysorbate 80 concentration on the intranasal absorption of TMPP

The above study demonstrated that intranasal administration significantly increased TMPP concentration in both plasma and brain. In this study, by using the same formulation, influence of polysorbate 80 concentration, 0.5%, 1.0%, 2.0%, and 5.0%, on the intranasal absorption of TMPP was investigated. The concentration-time profiles of TMPP in plasma are shown in Fig. 2a. The corresponding pharmacokinetic parameters are summarized in Table 2. Compared to the control group, polysorbate 80 addition did not change the T_{max}, but C_{max} and $AUC_{0–120}$ increased remarkably, indicating polysorbate 80 has no effect on absorption rate but could significantly enhance the absorption extent of TMPP in the nasal cavity. It was also noted that the increase of C_{max} and $AUC_{0–120}$ in plasma was polysorbate 80 concentration dependent; when polysorbate 80 concentration increased from 0.5 to 2.0%, the $AUC_{0–120}$ of TMPP in the plasma increased from 394.2 ± 21.3 to 582.9 ± 23.8 μg min/mL, with C_{max} from 16.7 ± 2.26 to 38.2 ± 1.7 μg/mL. Along with the improved absorption of TMPP, the absolute bioavailability was also significantly enhanced under the effect of polysorbate 80 up to 75%. However, further increasing polysorbate 80 concentration to 5.0% caused no further change in C_{max}, $AUC_{0–120}$, and absolute availability, with no statistical difference observed between 2 and 5% polysorbate 80 group, indicating 2% polysorbate 80 was the maximum concentration required to enhance TMPP absorption.

Effect of polysorbate 80 concentration on the distribution of TMPP in the brain

To test whether polysorbate 80 could be used as a messenger drug to increase drug distribution in the brain, first of all, effect of polysorbate 80 concentration on the brain distribution of TMPP after intranasal administration was investigated. As shown in Fig. 2b, similar trend was observed as that in the plasma profile, and

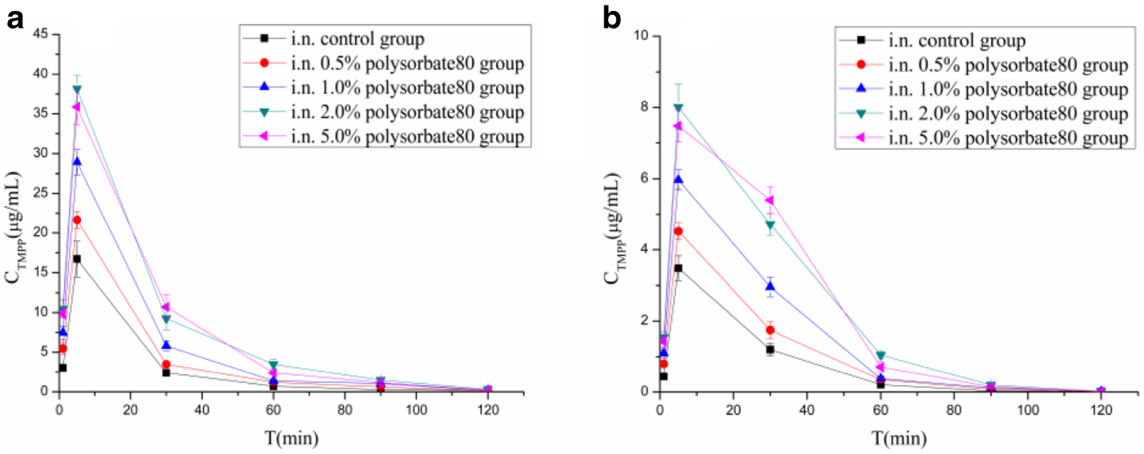

Fig. 2 Mean TMPP concentration-time profiles in plasma (**a**) and brain (**b**) after i.n. administration of formulations containing 0.5–5% polysorbate 80 in rats, compared with the control group (means ± SDs, $n = 5$)

polysorbate 80 addition increased the C_{max} and $AUC_{0–120}$ of TMPP in the brain significantly, without influence on the T_{max} value. In agreement with the plasma data, the best brain absorption enhancing effect was observed at 2% polysorbate 80 concentration; further increasing polysorbate 80 concentration to 5% caused no statistical improvement in both C_{max} and $AUC_{0–120}$ compared to the 2% group ($p > 0.005$). This result indicated polysorbate 80 indeed could enhance drug distribution in the brain in a concentration-dependent manner, with the best effect achieved at 2% concentration.

Effect of polysorbate 80 concentration on the brain targeting efficiency of TMPP

The above study demonstrated that polysorbate 80 could enhance TMPP concentration both in plasma and in brain after intranasal administration. Since TMPP can not only transport directly to the brain via the nose, the amount of drug absorbed in the plasma can also be redistributed into the brain from the systemic circulation via the BBB; therefore, influence of polysorbate 80 concentration on brain targeting effect needs further investigation. As shown in Table 2, polysorbate 80 concentration had no significant influence on brain targeting efficiency and drug targeting index of TMPP; however, drug direct transport percentage from the nose to brain was polysorbate 80 concentration dependent. The DTP value decreased with the increase of polysorbate 80 concentration, from 79.68% without polysorbate 80 to 63.31% at 2% polysorbate 80 concentration; further increasing polysorbate 80 concentration to 5% caused no statistical change of DTP.

Discussion

In this study, it was demonstrated that TMPP could penetrate BBB after systemic circulation; this is in agreement with the

Table 2 Phamacokinetic parameters in plasma and brain after i.n. administration of TMPP formulations containing different polysorbate 80 concentrations (means ± SDs, $n = 5$)

Parameters	i.n. control group	0.5% polysorbate 80	1.0% polysorbate 80	2.0% polysorbate 80	5.0% polysorbate 80
$(AUC_{0–120})_{plasma}$ (μg min/mL)	322.81 ± 14.2	394.2 ± 21.3*	448.5 ± 11.4*	582.9 ± 23.8*	571.7 ± 28.1*
$(AUC_{0–120})_{brain}$ (μg min/mL)	85.4 ± 3.6	95.2 ± 8.3*	110.8 ± 5.4*	153.9 ± 15.7*	145.8 ± 9.2*
$(C_{max})_{plasma}$ (μg/mL)	16.7 ± 2.26	21.7 ± 1.1*	28.9 ± 1.6*	38.2 ± 1.7*	35.8 ± 2.2*
$(C_{max})_{brain}$ (μg/mL)	3.5 ± 0.4	4.52 ± 0.2*	6.0 ± 0.3*	6.0 ± 0.3*	7.5 ± 0.5*
$(T_{max})_{plasma}$ (min)	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0
$(T_{max})_{brain}$ (min)	5 ± 0	5 ± 0	5 ± 0	5 ± 0	5 ± 0
$(MRT_{0–120})_{plasma}$ (min)	15.6 ± 0.2	17.4 ± 0.9	18.3 ± 0.3*	21.1 ± 1.2*	20.1 ± 0.9*
BA (%)	41.68 ± 1.83	50.90 ± 2.75*	57.91 ± 1.47*	75.26 ± 3.07*	73.81 ± 3.63*
DTE	0.265 ± 0.02	0.242 ± 0.03	0.247 ± 0.02	0.264 ± 0.04	0.255 ± 0.03
DTI (%)	490.7 ± 37.0	448.1 ± 55.6	457.4 ± 37.0	488.9 ± 74.0	472.2 ± 55.6
DTP (%)	79.68 ± 0.89	75.20 ± 1.34*	71.80 ± 0.72*	63.31 ± 1.50*	64.01 ± 1.77*

*$p < 0.05$, compared to i.n. control group

BA, absolute bioavailability

report indicating that TMPP has appreciable BBB penetrability [25, 26]. After intranasal drug delivery, brain targeting efficiency of TMPP was increased by 490.7%, implying that except for drug redistribution to the brain from systemic circulation via BBB, part of the drug can be transferred to the brain via the nasal route; similar phenomenon was observed for some other drugs [24, 27, 28]. Based on the direct drug transport percentage, 79.68% of the TMPP was transported directly to the brain, which might be absorbed via the olfactory region, trigeminal nerve pathway, or lymphatic pathway [4], contributed to the physiological structure and properties of the nasal cavity. And the remaining part of TMPP might be absorbed via the respiratory region to enter the systemic circulation and then partly redistributed in the brain. On the other hand, the higher nose-to-brain transport ratio of TMPP might also be attributed to the method of administration, while the rats were anesthetized and laid at a supine position during drug administration, which is advantageous for drug deposition to the olfactory region during drug application. Moreover, it should be indicated that the olfactory region in rats covered 50% of the nasal epithelium area, where it is 8% in human beings [29]. Therefore, compared with human beings, rats can better absorb drugs at the olfactory area. In addition, it was noted that compared to the oral route, the bioavailability of TMPP was significantly improved after intranasal drug delivery, further indicating that intranasal route is an effective way to improve the absorption of drugs.

The function of polysorbate 80 on drug absorption was analyzed. It was demonstrated that the addition of polysorbate 80 improved the systemic absorption of TMPP significantly and the absolute bioavailability increased with the increase of polysorbate 80 concentration in the range of 0.5–2.0% (AUC_{plasma} = 128.7C + 324.49, $R = 0.999$, $n = 4$). However, as the concentration further increased from 2.0 to 5.0%, the absorption of TMPP reached saturation and even slightly decreased. Similarly, Kaneda et al. found that after oral administration, the uptake percentage of salicylic acid, quinine hydrochloride, and metoclopramide hydrochloride decreased when polysorbate 80 concentration increased from 3.0 to 5.0%, from 1.0 to 3.0%, and from 1.0 to 3.0%, respectively [30], indicating that polysorbate 80 as an absorption enhancer; its optimal concentration is administration route and drug type dependent. About the possible absorption enhancing mechanism of polysorbate 80, there are different reports. Polysorbate 80 consists of polyoxyethylene and intermediate hydrocarbon chain, which carries both lipophilic and hydrophilic properties. The structural characteristics of polysorbate 80 imparted it to be able to solubilize and disorganize the intercellular lipids [31], which could increase the fluidity of the nasal mucosa. Moreover, polysorbate 80 might allocate between lipid and protein domains, which could increase drug permeability by disturbing the cell membrane [32]. It was also found that paracellular drug transport was improved when Caco-2 cell monolayers were exposed to polysorbate 80 solution for 3 h, indicating polysorbate 80 has the absorption enhancing effect partly due to loosening the tight junction [33, 34].

Moreover, to further test whether polysorbate 80 can be used as a brain message guider to improve drug distribution in the brain, influence of polysorbate 80 concentration on TMPP distribution in the brain was explored. It was noted that similar to the influence on systemic absorption, brain distribution of TMPP increased with the increase of polysorbate 80 concentration and the highest brain absorption was observed at polysorbate 80 concentration 2%; there was linear relationship between AUC_{brain} and polysorbate 80 concentration up to 2% (AUC_{brain} = 35C + 80.7, $R = 0.988$, $n = 4$), implying polysorbate 80 has the capacity to enhance drug distribution in the brain. To further elucidate whether the enhanced drug concentration in the brain is due to the increased drug concentration gradient between plasma and brain, or the guiding effect of polysorbate 80, drug brain targeting efficiency (DTE) and drug targeting index (DTI) were calculated. No statistical difference in DTE value was found between polysorbate 80 with and without groups, and DTI decreased slightly in polysorbate 80 0.5% and 1% groups, and recovered in polysorbate 80 2% group. Thus, it seems that the improved brain distribution of TMPP is partly owing to the increased drug concentration in the plasma. And the role of brain message guider is not significant while the polysorbate 80 is coadministered with the drug in solution. Similarly, it was reported that no uptake of doxorubicin into the brain was observed after intravenous administration of doxorubicin solution in 1% polysorbate 80 [11].

Taking into account that intranasal drug delivery can enhance drug distribution into the brain via different routes, the influence of polysorbate 80 concentration on nose-to-brain direct transport percentage (DTP) was calculated and compared. A liner correlation between DTP value and polysorbate 80 concentration up to 2% (DTP = − 8.10C + 79.58, $R = 0.999$, $n = 4$) was found, indicating that the addition of polysorbate 80 decreased the nose-to-brain direct transport percentage in a polysorbate 80 concentration-dependent manner. Probably due to the significantly enhanced drug concentration in the plasma and drug redistribution from plasma to the brain, the total drug brain targeting efficiency (DTE) was less influenced after intranasal drug delivery.

The above study indicated 2% polysorbate 80 is sufficient to increase TMPP concentration in both plasma and brain via intranasal route; however, the potential cytotoxicity of 2% polysorbate 80 on the nasal mucosa is also a main concern. Fortunately, it was reported that when the concentration of polysorbate 80 was no more than 5%, no substantial cilium toxicity was observed in the toad palate ciliotoxicity assay [35]. Thus, 2% polysorbate 80 might be safe for the nasal cavity. Still, the safety profile after long-term use needs to be further investigated in the near future.

Conclusions

In this study, the feasibility of using polysorbate 80 to enhance drug distribution to the brain after intranasal drug delivery was

investigated. It was demonstrated that, compared with intravenous administration, brain targeting efficiency of TMPP was improved remarkably by intranasal drug delivery contributed to nose-to-brain direct transport pathways. After intranasal administration, TMPP concentration in both the plasma and brain increased linearly with the increase of polysorbate 80 concentration up to 2%. Based on DTE, DTI, and DTP data, polysorbate 80 addition decreased nose-to-brain direct transport of TMPP but the total brain targeting efficiency was unchanged. In summary, polysorbate 80 can promote TMPP distribution in the brain by increasing drug systemic absorption and then enhanced passive transport of TMPP through the BBB, with the nose-to-brain direct transport percentage decreased to some extent.

Compliance with ethical standards

All institutional and national guidelines for the care and use of laboratory animals were followed.

Declaration The experiments comply with the current laws of China.

Conflict of interest The authors declare that they have no conflict of interest.

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