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RESEARCH PAPER

Direct Drug Delivery of Low-Permeable Compounds to the Central Nervous System Via Intranasal Administration in Rats and Monkeys

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ABSTRACT

Purpose Intranasal administration enhances drug delivery to the brain by allowing targeted-drug delivery. Here, we investigated the properties that render a compound suitable for intranasal administration, and the differences between rodents and non-human primates in delivery to the brain.

Methods The delivery of 10 low-permeable compounds to the brain, including substrates of efflux drug transporters expressed in the blood-brain barrier (didanosine, metformin, zolmitriptan, cimetidine, methotrexate, talinolol, ranitidine, atenolol, furosemide, and sulpiride) and two high-permeable compounds (ropinirole and midazolam) was evaluated following intranasal and intravenous administration in rats. Six of the 12 compounds (metformin, cimetidine, methotrexate, talinolol, sulpiride, and ropinirole) were also evaluated in monkeys, which have a similar nasal cavity anatomical structure to humans.

Results In rats, most of the low-permeable compounds displayed an obvious increase in the brain/plasma concentration ratio (K_p) by intranasal administration (despite their substrate liability for efflux drug transporters); this was not observed with the high-permeable compounds. Similarly, intranasal administration increased K_p for all low-permeable compounds in monkeys.

Conclusions Compound permeability is a key determinant of K_p increase by intranasal administration. This route of administration is more beneficial for low-permeable compounds and enhances their delivery to the brain in rodents and non-human primates.

KEY WORDS central nervous system · drug delivery · intranasal administration · membrane permeability

ABBREVIATIONS

AUC	Area under the plasma concentration-time curve
BBB	Blood-brain barrier
BCRP	Breast cancer resistance protein
CNS	Central nervous system
K_p	Tissue/plasma concentration ratio
LC/MS/MS	Liquid chromatography/tandem mass spectrometry
P-gp	P-glycoprotein

INTRODUCTION

The central nervous system (CNS) is a crucial component of the human body, consisting of the brain and the spinal cord. Many diseases, including Parkinson's disease, schizophrenia, and depression, are related to dysfunctions in the CNS. Despite the extensive efforts to develop therapeutic agents to cure CNS diseases, the therapeutic efficacy of systemic drug administration is often limited owing to the blood-brain barrier (BBB) (1). This barrier prevents most foreign substances from entering the brain when present in the circulating blood (2). To overcome this challenge, intranasal administration has been proposed as an attractive option to potentially provide the direct delivery of drugs via the nose-to-brain route, and a number of studies have been conducted with many different small and large molecules (3). While most of the investigations are currently in preclinical or early clinical stages, some

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human clinical studies suggest that the intranasal administration is a promising method for drug delivery to the brain (4). Craft *et al.* investigated intranasal administration of insulin for Alzheimer's disease and examined its efficacy in clinical studies on human volunteers (5). The study was conducted on 104 adults for approximately 4 years, and the results showed a significant improvement in memory upon administration of a 20 IU dose of insulin. Moreover, the author did not observe any treatment-related adverse effect in volunteers, even after long-term treatment.

While the exact mechanisms underlying intranasal drug delivery to the CNS are not fully understood, an accumulating body of evidence shows that pathways involving nerves connecting the nasal passage to the brain are important (6). This intracellular pathway begins with endocytosis in olfactory and trigeminal nerves, followed by axonal transport to their synaptic clefts where the drug is exocytosed (1). This trans-synaptic process is repeated by neurons, thereby distributing the drug to the other brain regions. In addition, pathways involving the vasculature, cerebrospinal fluid, and lymphatic system have been implicated in the transport of molecules from nasal cavity to the CNS (6). The extracellular mechanism involves the drug entering the paracellular space and being transported to the lamina propria (1). From the lamina propria, the drug is transported through the perineural space to the subarachnoid space. This movement is mediated by a variety of forces, including bulk flow and the perivascular pump (1). It is likely that a combination of these pathways is responsible, although one pathway may predominate, depending on the properties of the therapeutic, the characteristics of the formulation, and the delivery device used (6).

Although the effectiveness of intranasal administration in drug delivery to the brain has been widely investigated, details into the ideal chemical profile of compounds that target the brain via intranasal administration are limited. Sakane *et al.* reported the relationship between direct drug transport from the nasal cavity to cerebrospinal fluid (CSF), and the molecular weight in rats using fluorescein isothiocyanate-labeled dextran (FD) with various molecular weights. The results indicated that the transport of FDs to the CSF was inversely correlated with molecular weight (7). The researchers also showed that drug concentration in the CSF after intranasal administration increased with the lipophilicity of the drugs and conformed to the pH partition theory when some sulfonamides were used as model drugs (8,9). However, these authors did not investigate drug delivery to the brain tissue by intranasal administration. The involvement of drug transporters in nasal drug delivery has also been discussed (10–13), although further characterization is required. P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) are the efflux drug transporters expressed at the BBB (14). These proteins may reduce targeting to the brain during intranasal administration via active efflux of absorbed drug in brain. In terms of pharmacokinetics, we anticipated that the brain distribution of a compound with low BBB permeability is more likely to be improved by direct targeting via the nose-to-brain route. This is because the high permeability compound would display instantaneous equilibrium in the drug concentration between the brain and the systemic compartment. Therefore, our first aim was to investigate the relationship between the brain targeting effects via intranasal administration and the compound profiles in terms of membrane permeability and substrate liability of efflux drug transporters.

Despite the fact that the increase in brain delivery by intranasal administration was observed for several compounds in rodents (15–18), no quantitative data indicating brain targeting by the nose-to-brain route in humans have been reported. Consequently, the application of intranasal administration for brain targeting in clinical setting is still a current debate (3,19–21). One of the main uncertainties of translation from rodents to humans is derived from the large anatomical difference in the nasal cavity and brain between rodents and humans. Non-human primates, however, have a similar anatomical nasal structure to humans (22). This suggests that non-human primates can be used as an animal model for translational research on the pharmacokinetics of the nose-to-brain route. Our second aim was therefore to evaluate brain targeting by intranasal administration in monkeys to evaluate the application of intranasal administration across species.

In this study, 10 low-permeable compounds including efflux drug transporter substrates and two high-permeable compounds were administered to rats intravenously or intranasally. The concentration-time profiles in the plasma and brain were measured. Since the olfactory nerve pathways and trigeminal nerve pathways have been proposed as crucial components of intranasal delivery (6,23), the concentrations of the olfactory bulb, olfactory tract, and trigeminal nerve were also evaluated. The ratio of brain to plasma concentration (K_p) was compared between the intranasal and intravenous administration routes ($K_{p,in}/K_{p,iv}$) to establish an index for brain targeting by the intranasal route. Similar to rats, five low-permeable compounds and one high-permeable compound were administered to monkeys either intravenously or intranasally. The concentrations in plasma, olfactory bulb, olfactory tract, trigeminal nerve, and the rest of the brain were measured to evaluate the $K_{p,in}/K_{p,iv}$ in monkeys. Moreover, the relationship of $K_{p,in}/K_{p,iv}$ between the olfactory bulb, olfactory tract, trigeminal nerve, and the rest of the brain was investigated both in rats and monkeys. The difference in $K_{p,in}/K_{p,iv}$ between rats and monkeys in each brain region was also evaluated.

MATERIALS AND METHODS

Materials and Reagents

Didanosine, metformin, cimetidine, methotrexate, atenolol, furosemide, sulpiride, and midazolam were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ranitidine was purchased from APIN Chemicals Ltd. (Milton, United Kingdom). Zolmitriptan was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ropinirole was purchased from OChem Incorporation (Des Plaines, IL). Talinolol was synthesized by Takeda Pharmaceutical Co. Ltd. (Kanagawa, Japan). Methylcellulose was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). All other reagents were obtained from commercial sources.

In Vitro Permeability Assay

The parallel artificial membrane permeability assay (PAMPA) was conducted as follows: donor wells were filled with 200 μL PRISMA HT buffer (pH 7.4, pION Inc., Billerica, MA) containing 50 μmol/L of the test compound. The filter at the bottom of each acceptor well was coated with 4 μL of GIT-0 Lipid Solution (pION Inc.) and filled with 200 μL of Acceptor Sink Buffer (pION Inc.). The acceptor filter plate was placed on the donor plate and incubated for 3 h at room temperature. Following the incubation, the amount of test compound in both the donor and acceptor wells was measured via UV absorbance, using the SpectraMax® 190 microplate reader or SpectraMax® Plus 384 microplate reader (Molecular Devices, LLC., San Jose, CA).

Pharmacokinetics in Rat

Male Sprague-Dawley rats (7–8 weeks, approximately 300 g) were obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). Rats were maintained in a 12-h light/dark cycle and were granted free access to water and a commercial rodent diet (CE-2, CLEA Japan, Inc., Japan). All studies involving rats were approved by the Institutional Animal Care and Use Committee of Takeda Pharmaceutical Company. A cassette of six compounds (cassette 1: 1 mg/kg didanosine, 1 mg/kg zolmitriptan, 3 mg/kg ranitidine, 1 mg/kg atenolol, 1 mg/kg furosemide, and 0.1 mg/kg midazolam, or cassette 2: 1 mg/kg cimetidine, 1 mg/kg sulpiride, 1 mg/kg talinolol, 1 mg/kg methotrexate, 3 mg/kg metformin and 0.1 mg/kg ropinirole) was administered intravenously or intranasally ($n = 3$/time point) to rats. Dose and combination of test compounds were selected to minimize the interaction between compounds based on the inhibitory potential for P-gp and BCRP, as well as the target protein and its receptor occupancy. For intravenous administration, the compounds were dissolved in N,N-dimethylacetamide/saline (1/1, *v*/v) and administered via the tail vein of the rodents. For intranasal administration, the compounds were formulated as a suspension in 0.5% aqueous methylcellulose solution and filled in a catheter connected to a micropipette. The tip of the catheter was inserted into each nasal cavity followed by the release of the compound suspension by pipetting a volume of 0.05 mL/kg per nasal cavity. The rats were kept in a supine position and were conscious during administration. At 0.1, 0.25, 0.5, 1, and 2 h after administration, whole blood was collected from the abdominal aorta under anesthesia using isoflurane. The whole brain was perfused by 20 mL saline containing 1% heparin prior to the collection of the olfactory bulb, olfactory tract, trigeminal nerve, and the rest of the whole brain. Heparin was used as an anticoagulant for blood sampling. Plasma samples were prepared by centrifugation of blood at 4°C at 3000×*g* for 10 min. Brain samples were homogenized with 0.1 mol/L phosphate buffer solution at 4°C to prepare the 10 or 20% homogenate for the measurement of the concentration of the compound. All samples were stored at −80°C prior to the analysis.

Pharmacokinetics in Monkey

All procedures performed on monkeys were approved by the Institutional Animal Care and Use Committee of the contract research organization. Male cynomolgus monkeys (6–9 years old, 5–7 kg) were used for this study. Monkeys were maintained in a 12-h light/dark cycle and were granted free access to water and fed an approximately 108 g commercial primate diet (HF Primate 12 5K9J, Purina Mills, LLC, Shoreview, MN) once a day in the morning. A cassette of six compounds (1 mg/kg cimetidine, 1 mg/kg sulpiride, 1 mg/kg talinolol, 1 mg/kg methotrexate, 3 mg/kg metformin, and 0.1 mg/kg ropinirole; same combination in the cassette 2 in rat study) was administered intravenously and intranasally ($n = 4$/group). Cassette 2 was selected because it had the typical P-gp substrate (talinolol) and typical BCRP substrate (methotrexate) and was ideal to evaluate the effect of targeting substrates of efflux drug transporter to the brain via intranasal administration. The compounds were dissolved in N,N-dimethylacetamide/1,3-butanediol (1/1, *v*/v) for intravenous administration to the monkeys. For intranasal administration, the compounds were formulated as a suspension in 0.5% aqueous methylcellulose solution and administered around the cribriform plate area in each nasal cavity. The release was performed using a syringe attached to a rounded tip needle, whereby a volume of 100 μL per nasal cavity was administered. The monkeys

were kept at the dorsal position under anesthesia using isoflurane with nitrous oxide during the experiments. Blood, olfactory bulb, olfactory tract, trigeminal nerve, and the rest of the whole brain were isolated at 0.25 h after administration. Heparin was used as the anticoagulant for blood sampling. Plasma samples were prepared by centrifugation of blood at 4°C at 1700×*g* for 5 min. Brain samples were homogenized with 0.1 mol/L phosphate buffer solution at 4°C to prepare the 20% homogenate for quantification of the compound concentration. All samples were stored at −70 to −80°C prior to the analysis.

Quantification of Compound Concentrations by LC/MS/MS

All samples were precipitated with acetonitrile containing the internal standard (IS), alprenolol and diclofenac. The precipitated sample was centrifuged for 5 min at approximately 3000×*g*. The supernatants were diluted with the mobile phase and injected into the LC/MS/MS system. This system comprised a 20 AD-VP system (Shimadzu, Kyoto, Japan) and a triple quadrupole mass spectrometry detection API-5000 (AB Sciex, Framingham, MA), equipped with a turbo ion spray ionization source in the positive and negative ionization modes. Chromatographic separation was achieved with a reversed phase (C18) column (Shim-pack XR-ODS (2.2 μm, 2.0 × 30 mm, Shimadzu, Kyoto, Japan) at 50°C. For the positive ionization mode, the mobile phase consisting of 0.2% (*v*/v) formic acid in 0.01 mol/L ammonium formate (pH 3.0) (solvent A), and acetonitrile (solvent B), was delivered at a flow rate of 0.7 mL/min. For the negative ionization mode, the mobile phase, consisting of 0.01 mol/L ammonium acetate (pH 7.4) (solvent A), and acetonitrile (solvent B), was delivered at a flow rate of 0.7 mL/min. The analyte was eluted using a linear gradient of 95% solvent A / 5% solvent B to 5% solvent A / 95% solvent B. Detection was accomplished using multiple reaction monitoring in positive ionization mode (SRM $m/z = 315.1 \rightarrow 175.8$ for ranitidine, $m/z = 267.3 \rightarrow 145.2$ for atenolol, $m/z = 288.1 \rightarrow 58.1$ for zolmitriptan, $m/z = 237.2 \rightarrow 137.1$ for didanosine, $m/z = 326.0 \rightarrow 291.0$ for midazolam, $m/z = 253.3 \rightarrow 159.2$ for cimetidine, $m/z = 342.5 \rightarrow 112.4$ for sulpiride, $m/z = 364.3 \rightarrow 308.2$ for talinolol, $m/z = 455.5 \rightarrow 307.8$ for methotrexate, $m/z = 130.1 \rightarrow 71.1$ for metformin, $m/z = 261.2 \rightarrow 113.8$ for ropinirole, and $m/z = 250.3 \rightarrow 116.3$ for alprenolol (IS)), or in negative ionization mode (SRM $m/z = 328.9 \rightarrow 284.8$ for furosemide, $m/z = 294.0 \rightarrow 249.9$ for diclofenac (IS)). The Analyst software ™ (version 1.6.2, AB Sciex) was used for data acquisition and processing. The concentration of the compounds in each sample was calculated using a calibration curve generated from a set of calibration standards.

Pharmacokinetic Analysis

The area under the concentration *versus* time curve (AUC) in plasma or brain tissues was calculated by the linear trapezoidal method for the pharmacokinetics in rats. Calculations were conducted using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA).

RESULTS

Compounds Profiles

The delivery of 12 compounds (didanosine, metformin, zolmitriptan, cimetidine, methotrexate, talinolol, ranitidine, atenolol, furosemide, sulpiride, ropinirole and midazolam) was investigated in this study. The molecular weight and *in vitro* membrane permeability (Papp) of the compounds are summarized in Table I. Of the 12 compounds analyzed, metformin had the lowest molecular weight (129.2) and methotrexate had the highest (454.4). Based on the Papp results, ropinirole and midazolam were identified as high-permeable compounds (Papp >250 nm/s) while the other 10 compounds are low-permeable compounds (Papp <20 nm/s). Talinolol and methotrexate are typical substrates of the efflux transporters, P-glycoprotein (P-gp) and BCRP, respectively (27,28). Zolmitriptan and ranitidine are also known as substrates of P-gp (25,29) while furosemide is known as a substrate of BCRP (30). Cimetidine, metformin, and sulpiride, however, are recognized as substrates of P-gp and BCRP (24,26,31). The selected compounds have various profiles in terms of their

Table I Summary of Compound Profiles

	M.W.	Papp[a] (nm/s)	Transporter substrate liability		
			P-gp	BCRP	Reference
Didanosine	236.2	< 1	–	–	–
Metformin	129.2	< 1	+	+	(24)
Zolmitriptan	287.4	< 1	+	–	(25)
Cimetidine	252.3	< 2	+	+	(26)
Methotrexate	454.4	3	–	+	(27)
Talinolol	363.5	5	+	–	(28)
Ranitidine	314.4	6	+	–	(29)
Atenolol	266.3	< 9	–	–	–
Furosemide	330.7	< 11	–	+	(30)
Sulpiride	341.4	18	+	+	(31)
Ropinirole	260.4	268	–	–	–
Midazolam	325.8	308[b]	–	–	–

[a] Apparent permeability in PAMPA

[b] Referred to (32)

molecular weight, membrane permeability, and the involvement of efflux transporters in their pharmacokinetics.

Effect of Intranasal Administration in Rats

The concentration-time profiles of 12 compounds in the plasma, olfactory bulb, olfactory tract, trigeminal nerve, and the rest of the brain up to 2 h after intravenous and intranasal administrations at a dose of 0.1, 1, or 3 mg/kg in rats were evaluated (Supplemental Figs. 1, 2, 3, 4, and 5). K_p was calculated based on the AUC_{2h} to serve as an index of delivery to the brain. This value was compared between intranasal and intravenous administration ($K_{p,in}/K_{p,iv}$) to evaluate the increase in delivery when intranasal administration was used as the route of administration (Table II). The time profiles of tissue/plasma concentration ratio for each brain region were also evaluated to investigate the change in effect of intranasal administration with time on delivery to the brain (Fig. 1).

In the olfactory bulb, the $K_{p,in}$ values were clearly higher than the $K_{p,iv}$ for all compounds except ropinirole and midazolam. The olfactory bulb/plasma concentration ratios of didanosine, metformin, zolmitriptan, cimetidine, methotrexate, talinolol, ranitidine, atenolol, furosemide, and sulpiride following intranasal administration were continuously higher than those following intravenous administration up to 2 h (Fig. 1). The $K_{p,in}/K_{p,iv}$ of those compounds ranged from 37.8 to 265 (Table II), while the $K_{p,in}/K_{p,iv}$ of ropinirole and midazolam were < 2. Likewise, an increase in K_p by intranasal administration was observed when compared to that by intravenous administration in the olfactory tract. The olfactory tract/plasma concentration ratios of didanosine, metformin, zolmitriptan, cimetidine, methotrexate, talinolol, ranitidine, atenolol, furosemide, and sulpiride after intranasal administration were higher than those after intravenous administration up to 1 or 2 h, with the $K_{p,in}/K_{p,iv}$ values ranging from 4.51 to 84. Ropinirole and midazolam did not show the pronounced difference in olfactory tract/plasma concentration ratio between intranasal and intravenous administration with the $K_{p,in}/K_{p,iv}$ values <1.5. Similar to the olfactory tract, the trigeminal nerve/plasma concentration ratio increased by intranasal administration up to 1 or 2 h with $K_{p,in}/K_{p,iv}$ values >1.5 for most compounds, with the exception of talinolol, ropinirole, and midazolam. In the rest of the brain, the brain to plasma concentration ratios of didanosine, zolmitriptan, cimetidine, methotrexate, ranitidine, atenolol, furosemide, and sulpiride were higher after intranasal administration than after intravenous administration up to 1 or 2 h, with $K_{p,in}/K_{p,iv}$ values ranging from 1.74 to 27.6. Metformin, talinolol, ropinirole and midazolam did not show increased delivery to the rest of the brain by intranasal administration, and the $K_{p,in}/K_{p,iv}$ values of the compounds were < 1.5. Furosemide showed the highest $K_{p,in}/K_{p,iv}$ value in the olfactory tract, trigeminal nerve and the rest of the brain among the 12 compounds, followed by didanosine and ranitidine.

The relationships of $K_{p,in}/K_{p,iv}$ between the olfactory bulb and the olfactory tract, the olfactory tract and the rest of brain, and the trigeminal nerve and the rest of the brain were analyzed for the 12 compounds (Fig. 2). As shown in Fig. 2a, the degree of $K_{p,in}/K_{p,iv}$ in the olfactory bulb and in the olfactory tract were positively correlated with the exception of metformin. The $K_{p,in}/K_{p,iv}$ in the rest of the brain was positively correlating with that of the olfactory tract (Fig. 2b). In addition, the $K_{p,in}/K_{p,iv}$ in the trigeminal nerve showed a good correlation with that in the rest of the brain (Fig. 2c). The order of the degree of $K_{p,in}/K_{p,iv}$ among the brain regions was olfactory bulb > olfactory tract > trigeminal nerve ≈ the rest of the brain. The correlation of $K_{p,in}/K_{p,iv}$ in the rest of the brain with that of the olfactory tract or trigeminal nerve suggested that drug delivery occurred through the olfactory nerve pathways and/or the trigeminal nerve pathways after intranasal administration.

As shown in Supplemental Fig. 6, the $K_{p,in}/K_{p,iv}$ based on the AUC_{2h} correlated with the $K_{p,in}/K_{p,iv}$ calculated using the concentration at 0.25 h after administration. For 10–11 of the 12 compounds, the $K_{p,in}/K_{p,iv}$ values at 0.25 h were within the 2-fold difference of other $K_{p,in}/K_{p,iv}$ values based on the AUC_{2h} in the olfactory bulb, olfactory tract and rest of brain. Therefore, 0.25 h was selected as a representative time point for the monkey study.

Effect of Intranasal Administration in Monkeys

The optimal time point to investigate the effect of intranasal administration in monkeys was set to 0.25 h based on the data obtained from rats. The concentrations of metformin, cimetidine, methotrexate, talinolol, sulpiride, and ropinirole in the plasma, olfactory bulb, olfactory tract, trigeminal nerve, and the rest of the brain at 0.25 h after intravenous and intranasal administration at a dose of 0.1, 1, or 3 mg/kg in monkeys were evaluated (Table III). The tissue/plasma concentration ratio at 0.25 h (K_p at 0.25 h) was compared between intranasal and intravenous administration ($K_{p,in}/K_{p,iv}$ at 0.25 h). Similar to in rats, metformin, cimetidine, methotrexate, talinolol, and sulpiride showed a higher K_p value after intranasal administration, and in the olfactory bulb, these values ranged from 21.6 to 75.6. The $K_{p,in}/K_{p,iv}$ at 0.25 h in the olfactory bulb with ropinirole was 1.87 and a similar trend was observed in the olfactory tract, trigeminal nerve, and the rest of the brain. The $K_{p,in}/K_{p,iv}$ at 0.25 h in the olfactory tract for metformin, cimetidine, methotrexate, talinolol, and sulpiride ranged from 13.7 to 67.3, whereas that of ropinirole was 1.47. The $K_{p,in}/K_{p,iv}$ at

Table II Summary of Pharmacokinetic Parameters in Rats After Intravenous and Intranasal Administrations

			Plasma	Olfactory bulb	Olfactory tract	Trigeminal nerve	Rest of the brain
Didanosine	$AUC_{2h,iv}$	(ng·h/mL)	167	6.53	3.79	70.6	2.84
	$AUC_{2h,in}$	(ng·h/mL)	126	612	87.2	262	14.9
	$K_{p,iv}$			0.0391	0.0227	0.423	0.0170
	$K_{p,in}$			4.88	0.695	2.09	0.119
	$K_{p,in}/K_{p,iv}$			125	30.6	4.94	7.00
Metformin	$AUC_{2h,iv}$	(ng·h/mL)	1360	257	132	790	118
	$AUC_{2h,in}$	(ng·h/mL)	512	25,500	224	895	52.0
	$K_{p,iv}$			0.188	0.0968	0.579	0.0865
	$K_{p,in}$			49.8	0.437	1.75	0.101
	$K_{p,in}/K_{p,iv}$			265	4.51	3.02	1.17
Zolmitriptan	$AUC_{2h,iv}$	(ng·h/mL)	222	17.4	7.90	348	20.9
	$AUC_{2h,in}$	(ng·h/mL)	95.9	914	90.4	268	15.7
	$K_{p,iv}$			0.0784	0.0356	1.57	0.0941
	$K_{p,in}$			9.53	0.943	2.79	0.164
	$K_{p,in}/K_{p,iv}$			122	26.5	1.78	1.74
Cimetidine	$AUC_{2h,iv}$	(ng·h/mL)	326	10.8	6.55	181	5.37
	$AUC_{2h,in}$	(ng·h/mL)	114	157	23.0	186	6.76
	$K_{p,iv}$			0.0332	0.0201	0.556	0.0165
	$K_{p,in}$			1.38	0.202	1.63	0.0593
	$K_{p,in}/K_{p,iv}$			41.6	10.0	2.93	3.59
Methotrexate	$AUC_{2h,iv}$	(ng·h/mL)	962	37.0	17.0	319	20.0
	$AUC_{2h,in}$	(ng·h/mL)	340	493	108	443	31.9
	$K_{p,iv}$			0.0384	0.0177	0.331	0.0208
	$K_{p,in}$			1.45	0.318	1.30	0.0939
	$K_{p,in}/K_{p,iv}$			37.8	18.0	3.93	4.51
Talinolol	$AUC_{2h,iv}$	(ng·h/mL)	103	6.15	4.05	256	37.4
	$AUC_{2h,in}$	(ng·h/mL)	56.9	357	40.6	205	15.6
	$K_{p,iv}$			0.0597	0.0393	2.48	0.363
	$K_{p,in}$			6.27	0.713	3.60	0.274
	$K_{p,in}/K_{p,iv}$			105	18.1	1.45	0.755
Ranitidine	$AUC_{2h,iv}$	(ng·h/mL)	877	30.0	9.76	673	17.0
	$AUC_{2h,in}$	(ng·h/mL)	361	2560	283	865	42.4
	$K_{p,iv}$			0.0342	0.0111	0.767	0.0194
	$K_{p,in}$			7.09	0.783	2.39	0.117
	$K_{p,in}/K_{p,iv}$			207	70.5	3.12	6.03
Atenolol	$AUC_{2h,iv}$	(ng·h/mL)	602	45.8	19.2	474	21.2
	$AUC_{2h,in}$	(ng·h/mL)	188	1270	134	360	19.4
	$K_{p,iv}$			0.0761	0.0319	0.788	0.0352
	$K_{p,in}$			6.74	0.711	1.91	0.103
	$K_{p,in}/K_{p,iv}$			88.6	22.3	2.42	2.93
Furosemide	$AUC_{2h,iv}$	(ng·h/mL)	2490	7.76	4.50	125	2.00
	$AUC_{2h,in}$	(ng·h/mL)	680	372	103	357	15.1
	$K_{p,iv}$			0.00311	0.00181	0.0502	0.000803
	$K_{p,in}$			0.547	0.152	0.525	0.0222
	$K_{p,in}/K_{p,iv}$			176	84.0	10.5	27.6
Sulpiride	$AUC_{2h,iv}$	(ng·h/mL)	387	39.6	23.7	370	28.1
	$AUC_{2h,in}$	(ng·h/mL)	85.6	749	74.1	236	12.2
	$K_{p,iv}$			0.102	0.0612	0.955	0.0726

Table II (continued)

			Plasma	Olfactory bulb	Olfactory tract	Trigeminal nerve	Rest of the brain
	$K_{p,in}$			8.75	0.865	2.76	0.142
	$K_{p,in}/K_{p,iv}$			85.8	14.1	2.89	1.96
Ropinirole	$AUC_{2h,iv}$	(ng·h/mL)	14.7	61.4	74.2	64.4	68.2
	$AUC_{2h,in}$	(ng·h/mL)	6.98	44.0	43.6	41.3	42.3
	$K_{p,iv}$			4.19	5.06	4.39	4.65
	$K_{p,in}$			6.30	6.24	5.91	6.06
	$K_{p,in}/K_{p,iv}$			1.50	1.23	1.35	1.30
Midazolam	$AUC_{2h,iv}$	(ng·h/mL)	34.6	55.7	60.1	75.1	66.0
	$AUC_{2h,in}$	(ng·h/mL)	5.51	14.9	12.8	15.9	15.1
	$K_{p,iv}$			1.61	1.74	2.17	1.91
	$K_{p,in}$			2.70	2.32	2.88	2.74
	$K_{p,in}/K_{p,iv}$			1.68	1.33	1.33	1.43

Mean ($n = 3$ / time point), K_p; Ratio of AUC in tissue to plasma

0.25 h in the trigeminal nerve was >1.5 for all compounds except ropinirole. In the rest of the brain, the $K_{p,in}/K_{p,iv}$ values at 0.25 h for metformin, cimetidine, methotrexate, talinolol, sulpiride and ropinirole were 5.85, 12.0, 28.4, 9.96, 10.9, and 0.906, respectively. For all compounds except ropinirole, there was a clear increase in brain K_p by intranasal administration compared to intravenous administration. Methotrexate showed the highest $K_{p,in}/K_{p,iv}$ at 0.25 h in all brain regions.

Comparison of $K_{p,in}/K_{p,iv}$ Between Rats and Monkeys

The $K_{p,in}/K_{p,iv}$ at 0.25 h in the olfactory bulb, the olfactory tract, trigeminal nerve, or the rest of the brain was compared between rats and monkeys for the compounds, metformin, cimetidine, methotrexate, talinolol, sulpiride, and ropinirole (Fig. 3). In the olfactory bulb and trigeminal nerve, the $K_{p,in}/K_{p,iv}$ in monkeys was lower than that observed in rats for all the compounds. However, the order of the $K_{p,in}/K_{p,iv}$ values among the six compounds was similar between rats and monkeys (Fig. 3a). In the olfactory tract, the $K_{p,in}/K_{p,iv}$ values in rats and monkeys were comparable and a good correlation was also observed (Fig. 3b). In the rest of the brain, monkeys showed higher $K_{p,in}/K_{p,iv}$ than rats for metformin, cimetidine, methotrexate, talinolol, and sulpiride (Fig. 5C). For metformin and talinolol, the $K_{p,in}/K_{p,iv}$ values in the rat brain were < 1.5, while those in the monkey brain were 5.85 and 9.96, respectively. Overall, the order of the $K_{p,in}/K_{p,iv}$ values in the olfactory bulb, olfactory tract, trigeminal nerve, and the rest of the brain was similar between rats and monkeys. The degree of $K_{p,in}/K_{p,iv}$ in the rest of the brain in monkeys was higher than in rats, whereas the $K_{p,in}/K_{p,iv}$ values in the olfactory bulb and trigeminal nerve were lower in monkeys than in rats.

DISCUSSION

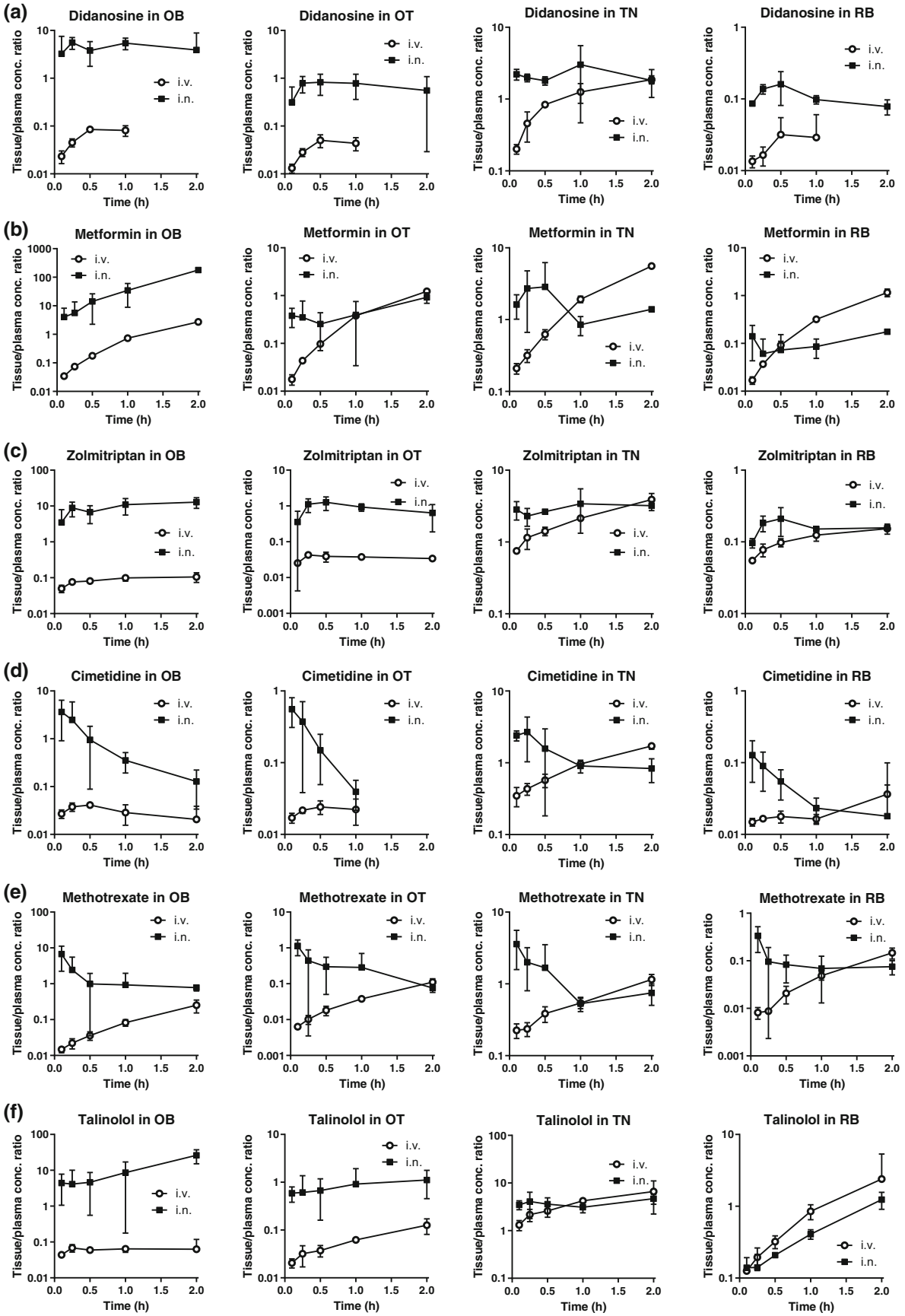
In this study, we investigated the effect of intranasal administration on direct drug delivery to the brain in rats and monkeys using compounds with various profiles (membrane permeability and efflux drug transporter substrate liability). As shown in Table II, the compounds with low membrane permeability showed higher $K_{p,in}/K_{p,iv}$ compared to the high-permeable compounds in the olfactory bulb, olfactory tract, trigeminal nerve, and rest of the brain of rats. A similar trend was also confirmed in monkeys as described in Table III. The $K_{p,in}/K_{p,iv}$ at 0.25 h of five low-permeable compounds were > 5 in the rest of the brain whereas that of the high-permeable compound ropinirole was 0.906. This is the first study to focus on the relationship between membrane permeability of a compound and the impact of intranasal administration on brain delivery. It was demonstrated that low-permeable compounds are more suited to intranasal administration than high-permeable compounds. Our results suggested that the low-permeable compounds could have more significant benefit of brain delivery via nose-to-brain absorption than high-permeable compounds. This might be due to their low brain delivery from the systemic route after systemic administration. Interestingly, talinolol and methotrexate, known as the typical substrates of P-gp and BCRP, respectively, showed obvious increases in K_p in the olfactory bulb, olfactory tract, trigeminal nerve, and the rest of the brain by intranasal administration in both rats and monkeys, with talinolol in rat trigeminal nerve

and rest of the brain ($K_{p,in}/K_{p,iv} < 1.5$) being the only exception. Other P-gp substrates, zolmitriptan, cimetidine, ranitidine, and sulpiride, also showed a higher K_p after intranasal administration in rats. Talinolol had higher $K_{p,iv}$ in the rest of the brain in rats (0.363) compared to other P-gp substrates (<0.1). This may explain why talinolol did not show a clear increase in K_p in the rest of the brain by intranasal administration. P-gp and BCRP are efflux drug transporters expressed in the BBB and one of the major components restricting the distribution of small molecule from systemic circulation into the CNS (14). P-gp is also localized in the bovine olfactory and nasal respiratory mucosa (33), and could limit the nose-to-brain transport of substrates in mice and rats (12,13). Despite the potential negative impact of those efflux transporters on the delivery of compounds to the brain, our data suggest that intranasal administration could have a significant effect on brain targeting, even for the substrates of efflux drug transporters. This finding is consistent with a previous report by Shingaki *et al.*, in which the authors demonstrated that talinolol had a higher K_p in rat brain during intranasal infusion compared to intravenous infusion (34). This suggests that intranasal administration is a useful strategy for brain delivery of efflux transporter substrates. Our study consolidates this hypothesis by demonstrating other examples including the BCRP substrate, methotrexate, in rodents as well as non-human primates.

The correlation of $K_{p,in}/K_{p,iv}$ in the rest of the brain with that in the olfactory tract and trigeminal nerve in rats suggested that drugs were delivered to the brain via the olfactory nerve pathways and trigeminal nerve pathways after intranasal administration (Fig. 2). The olfactory region is located posteriorly on the roof of each nasal cavity just beneath the cribriform plate of the ethmoid bone, which separates the two nasal cavities from the brain. The intranasally administered drug may reach the olfactory bulb first by paracellular transport across the olfactory neurons, and then enter other brain regions through the olfactory tract by simple diffusion (35). The trigeminal nerve, which innervates the respiratory and olfactory epithelium of the nasal passages and enters the pons, would serve as another important pathway connecting the nasal passage to the CNS (6). The trigeminal nerve enters the CNS from the respiratory epithelium of the nasal passages through the two sites, the anterior lacerated foramen and the cribriform plate near the olfactory bulbs. These pathways create entry points into both caudal and rostral brain areas following intranasal administration. For zolmitriptan, atenolol, and sulpiride, although the olfactory tract/plasma concentration ratio after intranasal administration was approximately 10-fold higher than that after intravenous administration up to 2 h, the increase in the tissue/plasma concentration ratio by intranasal administration in the rest of the brain was not sustained for 2 h. This was also similar in the trigeminal nerve as shown in Figs. 1c, h and j. Thus, the trigeminal nerve pathways may be a major component of nose-to-brain delivery of those compounds. Ranitidine showed a clear increase in tissue/plasma concentration ratio via the intranasal administration route in the rest of the brain and olfactory bulb up to 2 h. This was not observed in the trigeminal nerve, suggesting that the olfactory neuron pathways mainly contribute to the direct brain delivery of ranitidine. As reported in other literatures (6,35), our results have demonstrated that both the olfactory nerve pathway and trigeminal nerve pathway are involved in nose-to-brain drug delivery via the intranasal administration route.

Fig. 1 Time profiles of the ratio of tissue concentration to plasma concentration in rats after intravenous (open circle) or intranasal administration (closed square) (mean ± S.D., $n = 3$ / time point). OB; olfactory bulb, OT; olfactory tract, TN; trigeminal nerve, and RB; rest of the brain. When brain concentration was below the quantitation limit, the tissue/plasma concentration ratio was calculated to be 0 and not shown in the figure. The bottom of the error bar, which extends to a negative y-axis value, is not shown.

It has been reported that the intranasal route is composed of two pathways, one being intracellular while the other being extracellular (1). The intracellular pathway includes passive diffusion, adsorptive endocytosis, and receptor-mediated endocytosis. In contrast, the extracellular transport is mediated by multiple pathways, including bulk flow (1), and is faster (minutes to half an hour) than intracellular transport (hours to days) (36). In this study, the time to reach the maximum concentration (T_{max}) of test compounds in rat olfactory bulb, olfactory tract and the rest of brain after intranasal administration were within 1 h except for metformin (Supplemental Figs. 2, 3, and 5). These results suggest the rapid absorption of compounds via extracellular transport mechanism. On the other hand, the intracellular mechanism might involve in the transport of metformin based on the continuous increase in olfactory bulb concentration of metformin up to 2 h after intranasal administration. Metformin showed clear increase in K_p value in the olfactory bulb by intranasal administration ($K_{p,in}/K_{p,iv} = 265$), whereas K_p in the rest of the brain was similar between intranasal and intravenous administration ($K_{p,in}/K_{p,iv} = 1.17$). This discrepancy may be due to the slower transport mechanism. Increased delivery of metformin to the brain may be observed by extending the observation time > 2 h. In fact, the concentration of metformin in the rest of the brain after intranasal administration re-ascended after 1 h following the decline from 0.5 h to 1 h. If the intracellular pathway contributed to the transport of metformin, there is a possibility that organic cation/carnitine transporter 1 (OCTN1) was involved in the intracellular uptake of metformin.


(a)
Didanosine in OB
Didanosine in OT
Didanosine in TN
Didanosine in RB
(b)
Metformin in OB
Metformin in OT
Metformin in TN
Metformin in RB
(c)
Zolmitriptan in OB
Zolmitriptan in OT
Zolmitriptan in TN
Zolmitriptan in RB
(d)
Cimetidine in OB
Cimetidine in OT
Cimetidine in TN
Cimetidine in RB
(e)
Methotrexate in OB
Methotrexate in OT
Methotrexate in TN
Methotrexate in RB
(f)
Talinolol in OB
Talinolol in OT
Talinolol in TN
Talinolol in RB
Tissue/plasma conc. ratio
Time (h)
i.v.
i.n.

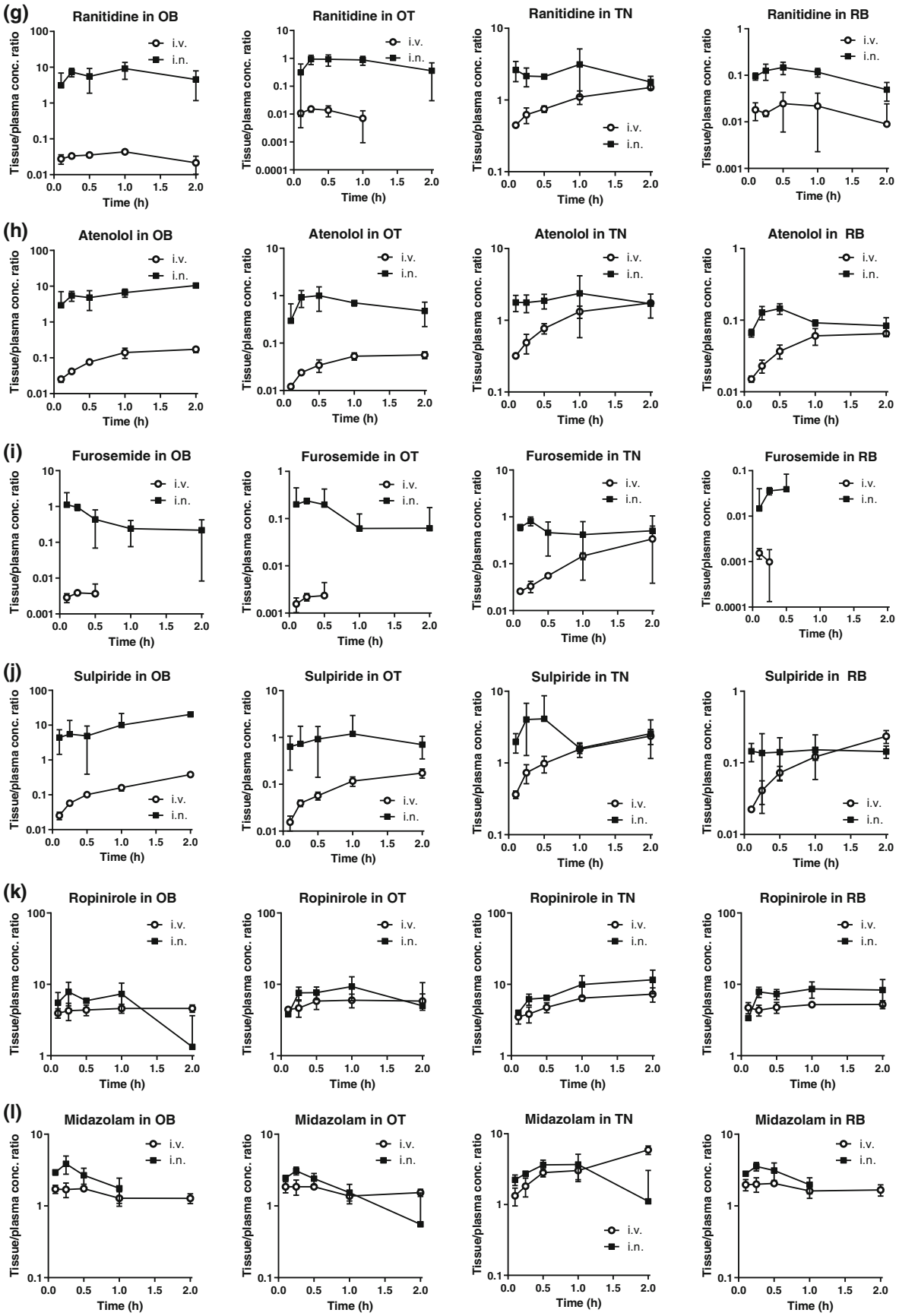


Fig. 1 (continued)

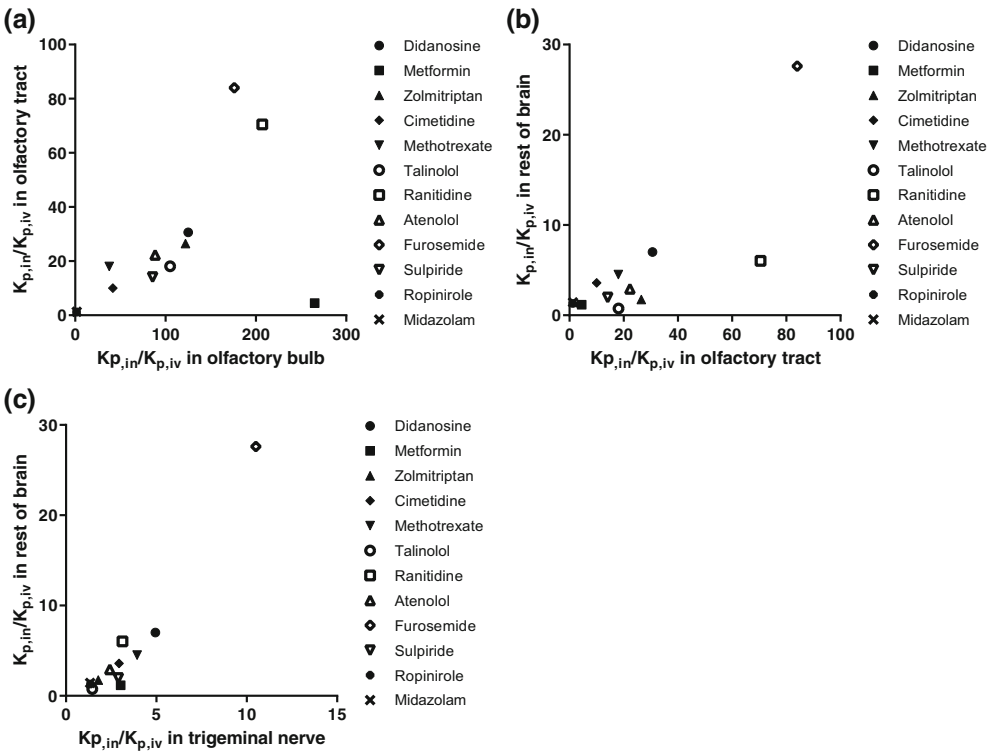


Fig. 2 The relationship of $K_{p,in}/K_{p,iv}$ between olfactory bulb and olfactory tract (**a**), olfactory tract and rest of the brain (**b**), and trigeminal nerve and rest of the brain (**c**) in rats.

OCTN1 is one of the uptake drug transporters functionally expressed in neuron and neural stem cells (37). Metformin is known to be a substrate of OCTN1 (38).

Rats and mice have been widely used in intranasal administration studies. However, given the difference in the anatomical structure of nose and brain between rodents and humans (22), the translation of the brain targeting effect via intranasal administration from rodent to humans is controversial (20). Monkeys and humans, however, have a similar anatomical structure of the nose-to-brain route. The olfactory epithelium, which could be an entrance for nose-to-brain delivery, covers approximately 10% of the nasal mucosal cavity in monkeys and humans. In rats however, 50% of the nasal mucosal cavity is occupied by the olfactory epithelium (22). Monkeys and humans also have a similar conchae complexity, a single scroll structure, while rodents, guinea pigs and sheep have a double scroll structure, and dogs and rabbits have a branching structure. The ratios of olfactory bulb volume to whole brain volume in rats, monkeys, and human were 4.65, 0.10 (observed data in this study) and 0.01% (39,40), respectively. These observations suggest that the use of monkeys as an animal model to project the delivery of compounds to the brain via intranasal administration in humans is more applicable than the use of rodents. However, the knowledge pertaining to the pharmacokinetics in monkey brain after intranasal dosing is relatively limited. Therefore, we elucidated the brain concentrations of several compounds after intranasal and intravenous administrations in monkey to evaluate the application of nose-to-brain targeting of small molecules in primates. Surprisingly, all poorly permeable compounds (metformin, cimetidine, methotrexate, talinolol and sulpiride) showed an obvious increase in K_p in the olfactory bulb, olfactory tract, trigeminal nerve, and the rest of the brain via the intranasal administration route in monkeys. Notably, this was observed despite the relatively small coverage of olfactory epithelium in their nasal cavity (Table III). To our knowledge, this is the first study to demonstrate the enhancement in brain delivery of small molecules via the intranasal administration route in non-human primates. Yamada *et al.* reported the enhancement of sleep-inducing effects in cynomolgus monkeys following intranasal administration of TS-002, an analog of prostaglandin D2, when compared to intravenous administration (41). This result suggested that intranasal administration increased the brain concentration of TS-002 in cynomolgus monkeys. However, the brain concentrations were evaluated only in rats, and the quantitative pharmacokinetics in monkeys was not reported.

The comparison of $K_{p,in}/K_{p,iv}$ at 0.25 h between rats and monkeys showed higher $K_{p,in}/K_{p,iv}$ in the olfactory bulb and trigeminal nerve in rats, comparable $K_{p,in}/K_{p,iv}$

Table III Summary of Pharmacokinetic Parameters in Monkeys After Intravenous and Intranasal Administration

			Plasma	Olfactory bulb	Olfactory tract	Trigeminal nerve	Rest of the brain
Metformin	$C_{0.25h,iv}$	(ng/mL)	6220 ± 1540	215 ± 122	178 ± 85	802 ± 686	76.0 ± 15.6
	$C_{0.25h,in}$	(ng/mL)	1550 ± 691	870 ± 316	599 ± 664	402 ± 98.8	90.1 ± 30.2
	$K_{p,iv}$			0.0328 ± 0.0102	0.0277 ± 0.00775	0.116 ± 0.0745	0.0123 ± 0.00104
	$K_{p,in}$			0.708 ± 0.458	0.378 ± 0.293	0.301 ± 0.126	0.0721 ± 0.0461
	$K_{p,in}/K_{p,iv}$			21.6 ± 15.5	13.7 ± 11.3	2.59 ± 1.99	5.85 ± 3.78
Cimetidine	$C_{0.25h,iv}$	(ng/mL)	778 ± 292	42.8 ± 27.3	29.7 ± 19.6	195 ± 180	8.18 ± 6.22
	$C_{0.25h,in}$	(ng/mL)	205 ± 82.4	321 ± 144	195 ± 207	117 ± 25.4	19.6 ± 5.84
	$K_{p,iv}$			0.0514 ± 0.0141	0.0360 ± 0.0146	0.234 ± 0.138	0.00982 ± 0.00458
	$K_{p,in}$			1.71 ± 0.679	0.935 ± 0.738	0.645 ± 0.247	0.118 ± 0.0755
	$K_{p,in}/K_{p,iv}$			33.3 ± 16.1	25.9 ± 23.0	2.76 ± 1.95	12.0 ± 9.50
Methotrexate	$C_{0.25h,iv}$	(ng/mL)	4180 ± 1050	68.1 ± 31.6	54.1 ± 12.5	244 ± 170	25.6 ± 1.39
	$C_{0.25h,in}$	(ng/mL)	592 ± 334	673 ± 525	593 ± 814	242 ± 85.0	68.2 ± 26.6
	$K_{p,iv}$			0.0157 ± 0.00453	0.0136 ± 0.00446	0.0654 ± 0.0485	0.00642 ± 0.00160
	$K_{p,in}$			1.18 ± 0.545	0.914 ± 0.964	0.497 ± 0.240	0.182 ± 0.173
	$K_{p,in}/K_{p,iv}$			75.6 ± 41.1	67.3 ± 74.3	7.59 ± 6.71	28.4 ± 27.9
Talinolol	$C_{0.25h,iv}$	(ng/mL)	441 ± 161	31.8 ± 6.27	30.0 ± 3.67	253 ± 159	9.48 ± 3.62
	$C_{0.25h,in}$	(ng/mL)	120 ± 83.1	254 ± 182	223 ± 300	118 ± 64.1	18.6 ± 4.01
	$K_{p,iv}$			0.0773 ± 0.0247	0.0747 ± 0.0288	0.668 ± 0.467	0.0253 ± 0.0152
	$K_{p,in}$			2.37 ± 1.13	1.89 ± 2.01	1.11 ± 0.335	0.252 ± 0.205
	$K_{p,in}/K_{p,iv}$			30.6 ± 17.6	25.3 ± 28.6	1.66 ± 1.26	9.96 ± 10.1
Sulpiride	$C_{0.25h,iv}$	(ng/mL)	1120 ± 134	48.5 ± 14.8	40.7 ± 10.2	299 ± 218	14.9 ± 3.09
	$C_{0.25h,in}$	(ng/mL)	203 ± 84.5	280 ± 144	199 ± 239	116 ± 28.9	21.9 ± 8.40
	$K_{p,iv}$			0.0433 ± 0.0124	0.0367 ± 0.0101	0.269 ± 0.183	0.0136 ± 0.00399
	$K_{p,in}$			1.46 ± 0.503	0.898 ± 0.826	0.627 ± 0.183	0.148 ± 0.135
	$K_{p,in}/K_{p,iv}$			33.7 ± 15.1	24.5 ± 23.5	2.33 ± 1.72	10.9 ± 10.4
Ropinirole	$C_{0.25h,iv}$	(ng/mL)	26.1 ± 4.89	104 ± 28.5	114 ± 26.4	88.3 ± 35.6	95.0 ± 25.1
	$C_{0.25h,in}$	(ng/mL)	14.8 ± 4.75	108 ± 43.9	97.0 ± 40.2	61.5 ± 13.4	49.3 ± 12.7
	$K_{p,iv}$			4.02 ± 0.936	4.55 ± 1.56	3.45 ± 1.29	3.78 ± 1.30
	$K_{p,in}$			7.51 ± 2.19	6.70 ± 1.79	4.49 ± 1.56	3.43 ± 0.367
	$K_{p,in}/K_{p,iv}$			1.87 ± 0.697	1.47 ± 0.639	1.30 ± 0.662	0.906 ± 0.326

Mean ± S.D. ($n = 4$), K_p; Ratio of concentration in tissue to plasma

in the olfactory tract, and higher $K_{p,in}/K_{p,iv}$ in the rest of the brain in monkeys. These results suggest that the drug could be delivered to the rest of the brain faster or more efficiently in monkeys compared to rats; this may be a result of the anatomical difference between rats and monkeys. Rats had relatively larger volume fractions of olfactory bulb, olfactory tract, and trigeminal nerve to the whole brain (4.65, 4.16, and 1.63%) than monkeys (0.10, 0.05, and 0.13%). The drugs administered via the nasal cavity might be able to reach the rest of the brain with higher availability in monkeys because of their smaller volume of olfactory and trigeminal nerve pathways compared to rats. Further studies will be required for a more quantitative comparison between rats and monkeys given the limited sampling time in the monkey study and the difference in dosing condition (*i.e.* monkeys were anesthetized for ease of administration). However, similar order/ranking of $K_{p,in}/K_{p,iv}$ among the compounds in rats and monkeys suggested that rats can be utilized for compound screening, to investigate the potential of brain delivery via the intranasal administration route. Concerning quantitative translation of nose-to-brain pharmacokinetics from animals to humans, one strategy is to develop the physiologically-based pharmacokinetic (PBPK) model that captures the intranasal pharmacokinetics in animals, and then translates the physiological parameters from the model to humans. Our next step is to investigate this approach using the data obtained in the present study. If the PBPK model can reproduce the intranasal pharmacokinetics in rats and monkeys, the projection of nose-to-brain delivery in humans will likely be more feasible.

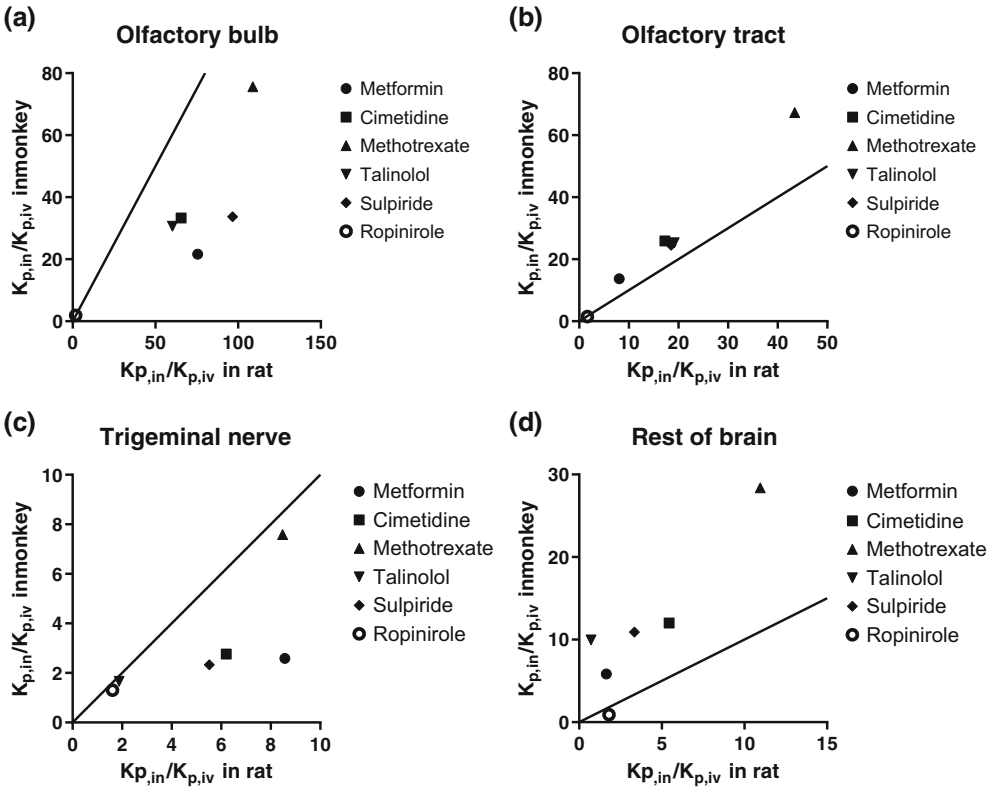


Fig. 3 The relationship of $K_{p,in}/K_{p,iv}$ at 0.25 h in rats and in monkeys for olfactory bulb (**a**), olfactory tract (**b**), trigeminal nerve (**c**), and rest of the brain (**d**). Solid line represents the line of unity.

Although the compounds with low membrane permeability could provide a larger benefit in direct brain targeting via intranasal dosing, this does not necessarily imply that we should aim to deliver low-permeable compounds via the intranasal route, since this may not always be the best approach for developing drugs targeting the CNS. It should be noted that even after intranasal administration, the K_p values in the rest of the brain of low-permeable compounds were much lower than those of high-permeable compounds (Table II and III). However, if the toxicity induced in non-CNS organs is the critical issue, the expansion of the K_p in brain compared to non-CNS organs via the intranasal administration route may mitigate the toxicity. In addition, when structural modifications aimed at improving CNS penetration from systemic compartment attenuates the pharmacological activity against the target protein, intranasal administration could be employed to maximize efficacy.

In conclusion, the present study demonstrated that the intranasal administration route could increase the delivery of low-permeable small molecules to brain in both rats and monkeys, despite their substrate liabilities of efflux drug transporters. Intranasal administration could be a promising option to rescue the compounds which fail to be sufficiently distributed to the brain from systemic circulation. The chemical options for CNS therapeutics could be expanded by incorporating the intranasal administration route as a drug discovery strategy. While further studies are necessary to investigate the translational approach from animals to humans, the clear increase in CNS distribution by intranasal administration in monkeys highlights the strong potential of nasal dosing in primates, to promote efficient drug delivery to the brain.

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All authors were employees of Takeda Pharmaceutical Company Limited when the study was performed. The authors declare that they have no conflict of interest.

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