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Research Article

Direct nose-to-brain delivery of diazepam via trigeminal nerve contributes to rapid seizure suppression in pentylenetetrazole-induced status epilepticus model rats

Kazutoshi Watanabe*, Soichiro Kimura, Yutaro Hazama, Yasunori Morimoto, Hideo Ueda

Laboratory of Hospital Pharmacy, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan

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*Address for Correspondence:

Kazutoshi Watanabe, Laboratory of Hospital Pharmacy, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan

Abstract

The purpose of our present study was to elucidate the involvement of the direct transfer to the brain after intranasal (*i.n.*) administration of diazepam (DZP), and to provide findings whether *i.n.* the administration could be used to obtain rapid onset of pharmacological action. We determined the blood and brain kinetics of DZP after administrations of *i.n.* and other routes, and the relationship between their concentrations and rapid seizure suppression effect; furthermore, and evaluated the distribution process of DZP to the brain. There was a negative connection between the plasma concentration and the amount of DZP delivery to the brain from the systemic circulation during the period that followed intravenous and intrarectal injection before the onset of seizure suppression. *I.n.* administration resulted in the seizure suppression time below the correlation curve: the seizure suppression effects were observed earlier than estimated despite the low plasma concentrations of DZP, suggesting the involvement of direct nose-to-brain delivery of DZP. The time to maximum concentration (T_{max}) in the forebrain, hindbrain, olfactory bulb (OB), trigeminal nerve (TN), and cerebrospinal fluid (CSF) after *i.n.* the administration was 3 min, which was shorter than the T_{max} of the plasma concentration. In fluorescence imaging using Rhodamin-B-base, the TN and the area of the vicinity of the thalamus had stronger fluorescence 1 and 3 min after *i.n.* administration, compared with the OB and CSF. In summary, direct brain delivery of DZP requires the TN and it was suggested that distribution to the vicinity of the thalamus via the TN may be connected to the quick seizure suppression effect.

Keywords: Status Epilepticus, Diazepam, Intranasal Administration, Nose-to-Brain, Trigeminal Nerve

1. INTRODUCTION

Status epilepticus (SE) is defined as a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms that lead to abnormally prolonged seizures [1]. Depending on the kind and length of seizures, persistent SE has the potential to result in long-term repercussions such as neuronal death, neuronal damage, and change of neural networks [1]. In general, SE is been treated with intravenous (*i.v.*) administration of benzodiazepines (BZPs) for rapid seizure control at medical institutions [2]. It reported that the incidence of SE is high in neonates associated with cardiopulmonary disease [3] and in the population aged 50 and over in developed countries [4-8]. There is a difficulty for prompt therapy by the *i.v.* administration of treatment drugs, because blood vessels in children and elder people are narrow for secure injection [9]. Therefore, intranasal (*i.n.*) delivery rather than *i.v.* injection is gaining attention in terms of quick and practical therapy both at home and in medical facilities. A drug administered intranasally is transferred, in part, into the brain parenchyma via the blood-brain barrier (BBB) after nasal absorption into the systemic circulation (indirect route), and transferred, in another part, directly from the nasal cavity to the brain parenchyma via the nose-to-brain (NTB) route (direct route) [10-12]. The NTB route may contribute to the rapid transport of medications to the brain parenchyma for an expedited start to their pharmacological effects. Drugs' transfer to the biomembrane and rate of transit through the membrane is determined by their partition coefficients (log P) [13]. Diazepam (DZP), one of the BZPs, is expected to show high transport properties to the brain through the BBB due to its relatively high lipophilicity, log P of 2.8 [14]. According to reports, DZP has a greater rate of brain transfer than BZPs like lorazepam and midazolam [15, 16], and it acts pharmacologically quickly in humans within 30 seconds of intravenous injection [17]. Kaurr et al. described, in their kinetic studies after *i.n.* administration of DZP in rabbits and rats that DZP was transported to the brain parenchyma mainly through the indirect route via BBB, but not through the direct route via the olfactory epithelium [18]. However, findings obtained by Stuart et al. in Wistar rats showed the time to maximum concentration (T_{max}) and the contribution ratio of the direct NTB route (DTP%) after *i.n.* administration of GW468816 (log P = 3.8) were ~1 min and 99.6% [19]. These findings imply that lipophilic medications, such as GW468816, can go directly from the nose to the brain, particularly in the initial stages following *i.v.* injection. However, elaborate studies providing the pharmacokinetics in blood and brain immediately after *i.n.* administration of DZP as well as the potential contribution of the direct NTB route are limited. Our current study's goals were to clarify the role of direct brain-to-nasal cavity transfer following *i.n* administered DZP and to determine whether such administration may be used to achieve a rapid beginning of the pharmacological activity. When comparing the relationship of DZP concentration in plasma and onset of pharmacological action after *i.n.* administration with those after *i.v.*, intrarectal (*i.r.*), and subcutaneous (*s.c.*) administrations, if the relationship after *i.n.* administration shift to shorten the time side of the action onset and the side of a lower DZP concentration in plasma than that after *i.v.*, *i.r.,* and *s.c.* administrations, the enhancement of the action can be attributed to direct transfer into the brain. When comparing DZP concentrations in plasma and brain tissues including the forebrain (FB), hindbrain (HB), olfactory bulb (OB), trigeminal nerve (TN), and cerebrospinal fluid (CSF) after *i.n.* administration with those after *i.v.* administration, if DZP concentration in the brain is a result of direct transfer via the NTB pathway, the T_{max} in the brain tissues should be shortened than those after *i.v.* administration.

2. MATERIALS AND METHOD

2.1 Reagent

DZP was purchased from FUJIFILM Wako Pure Chemical Industries, Ltd. (Japan). Acetonitrile and methanol for HPLC were purchased from Kanto Chemical Co., Inc. (Japan). Pentobarbital sodium (PEN) and pentylenetetrazole (PTZ) were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Rhodamine B base （RBB） was obtained from Sigma-Aldrich (USA).

2.2 Animals

Male Wistar rats (8 weeks old, body weight: 160 - 200 g) were purchased from Sankyo Lab Service Co., Ltd. (Japan). Rats were housed in 3–4/cage and had free access to food and water. Before the studies, animals fasted for 24 hours. The Life Science Research Center at Josai University followed the rules set forth by the Institutional Animal Care and Use Committee for all of the research, which was all carried out in compliance with those standards (JU19016, JU20019, JU21019, JU22021).

2.3 Pharmacokinetic studies

Rats were anesthetized with PEN (50 mg/kg) intraperitoneally (*i.p.*). DZP was given at a single dose of 0.5 mg/kg for *i.v.* and *i.n.* pharmacokinetic studies and at 1.0 mg/kg for the *i.r.* study. DZP was administered through the left jugular cannula, nasogastric tube, and anal tube for *i.v.*, *i.n.,* and *i.r.* administrations, respectively. Between 1 and 180 min following injection, blood samples (0.4 mL) were taken using a cannula inserted into the right jugular vein. Samples of blood were centrifuged (25°C, 8,000 rpm, 15 min) to obtain plasma (0.2 mL). Blood and CSF were taken from each rat at 1, 3, 5, 10, 30, and 60 min after DZP administration under varied circumstances to assess the DZP concentration profiles in brain tissues. Following systemic perfusion, the rats were killed and brain tissues including the FB, HB, OB, and TN were excised from each rat. Acetonitrile (3 mL) was added to 1.0 g of each tissue. The tissues were homogenized to obtain a mixed tissue fluid. The concentration of DZP in the brain tissues was ascertained using the supernatant that was collected after centrifugation (4°C, 12,800 rpm, 15 min). Plasma, CSF, and the supernatant of brain tissue samples were stored at −70°C until analysis.

2.4 Pharmacological studies

After *i.p.* administration of PTZ (120 mg/kg) to awake rats, rats in which tonic-clonic convulsions persisted for 5 min were used as PTZ-induced SE model rats. Following 90 min of DZP treatment using various administration techniques, the behavior of rats was studied. The time until the disappearance of convulsions after the DZP administration was defined as the convulsive suppression time. The ratio of rats who experienced convulsions to all rats who underwent testing is the convulsive suppression rate. The rank of the symptom observed was evaluated based on the severity classification of the Racine scale (Table 1) [20]. The seizure suppression time was defined as the period following the injection of DZP during which convulsions persisted. To show the relationship between seizure suppression time and plasma concentration, seizure suppression times when *i.v.* (1.0, 0.5, and 0.2 mg/kg), *i.n.* (2.0, 1.0, and 0.5 mg/kg), *i.r.* (4.0, 2.0, and 1.0 mg/mL) and subcutaneous (*s.c.*) (2.0 mg/kg) administrations of DZP were measured, and a blood sample was collected from the tail vein immediately after the seizure suppression and used to determine the plasma concentration.

Table 1: Classification of severe seizure symptoms in PTZ-induced SE model rats.

Scale	Seizure intensity
1	Sudden behavioral arrest and/or motionless staring
2	Facial jerking with muzzle or muzzle and eye
3	Neck jerks
4	Clonic seizure in a sitting position
5	Convulsions including clonic and/or tonic-clonic seizures while lying on the stomach and/or pure tonic seizures
6	Convulsions, including tonic-clonic and/or clonic seizures that include side-lying or uncontrolled jumping

2.5 *Ex vivo* fluorescence imaging

Anesthetized rats were administrated with RBB (0.5 mg/kg) under various conditions. Following RBB treatment, CSF was taken at 1, 3, 5, 10, 30, or 60 min. Each rat's OB, TN, and entire brain were then removed. The entire brain was separated from the nasal cavity toward the pharynx to reveal the left and right sides of the interior of the brain. Fluorescence images of collected brain tissues and CSF were acquired by the IVIS spectrum *in vivo* imaging system (PerkinElmer® Ltd., USA).

2.6 Quantification methods

The HPLC equipment consists of a UV-VIS detector (2489 UV/visible detector), column oven (Alliance column heater), and HPLC column (Mightysil RP-18, 5 mm, 4.6 mmid × 250 mm, Kanto Chemical Co., Ltd., Japan). The HPLC column was eluted with PBS, acetonitrile, and methanol (5:4:1) at a flow rate of 1.0 mL/min, the UV detector was set at a wavelength of 231 nm and the column temperature was maintained at 40°C.

2.7 Pharmacokinetic parameter analysis

The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (T_{max}) were determined from the plasma concentration profiles after DZP administration under various conditions. The trapezoidal rule was used to calculate the (AUC_{0-180}), or area under the plasma concentration-time curve. Furthermore, bioavailability (F) when *i.n.* or *i.r.* administration of DZP was calculated by the following equation (1):

$$F = \frac{AUC_{i.n.or\ i.r.}/D_{i.n.or\ i.r.}}{AUC_{i.v.}/D_{i.v.}} \times 100 \qquad eq.(1)$$

The maximum concentration in various brain tissues ($C_{max,\ brain}$) and the time to reach the maximum concentration in various brain tissues ($T_{max,\ brain}$) after DZP administration were determined from concentration profiles in various brain tissues. Moreover, the area under the concentration-time curve in various brain tissues ($AUC_{brain,\ 0-60}$) was determined by the trapezoidal method. Furthermore, the drug targeting efficiency for various brain tissues (DTE_{brain}) was derived from the following formula (2) [21].

$$DTE = \frac{(AUC_{brain}/AUC_{plasma})_{i.n.}}{(AUC_{brain}/AUC_{plasma})_{i.v.}} \quad eq.(2)$$

The direct transport percentage (DTP_{brain}), the index of direct transport from the nasal cavity into various brain tissues, was calculated from the following equations (3) and (4) [21].

$$DTP\ (\%) = \frac{B_{i.n.} - B_x}{B_{i.n.}} \times 100 \qquad eq.(3)$$

$$B_x = \frac{B_{i.n.}}{P_{i.v.}} \times P_{i.n.} \qquad eq.(4)$$

Student's *t*-test was used for comparison between two groups, and the Tukey-Kramer test was used for multiple comparisons. Two-sided $p < 0.05$ and $p < 0.01$ were considered statistically significant.

3. RESULT

3.1 Pharmacokinetic studies

3.1.1 Pharmacokinetic analysis of DZP after *i.v.* , *i.n.,* and *i.r.* administrations

Figure 1 and Table 2 show the plasma concentration-time profiles and the resultant pharmacokinetic parameters of DZP, respectively, after *i.v.*, *i.n.*, and *i.r.* administrations. C_{max} and T_{max} were 0.323 ± 0.016 μg/mL and 4.5 ± 0.5 min, respectively, for *i.n.* administration with 0.5 mg/kg dose, 0.227 ± 0.007 μg/mL, and 10.0 ± 0.0 min for *i.r.* administration with a 1.0 mg/kg dose (Table 2). Even administered dose was half in *i.n.* administration, C_{max} was 1.4-fold higher and T_{max} was shortened by half compared with those obtained by *i.r.* administration (Table 2). The calculated AUC_{0-180} values following *i.n.* (0.5 mg/kg) and *i.r.* administrations (1.0 mg/kg) were 13.7 ± 0.7 min · μg/mL and 9.9 ± 0.6 min · μg/mL, and the absolute bioavailability values were calculated as 20.8 ± 1.0% and 7.5 ± 0.4%, respectively (Fig. 1 and Table 2). These findings show that systemic DZP absorption following *i.n.* the injection is superior to that following *i.r.* administration.

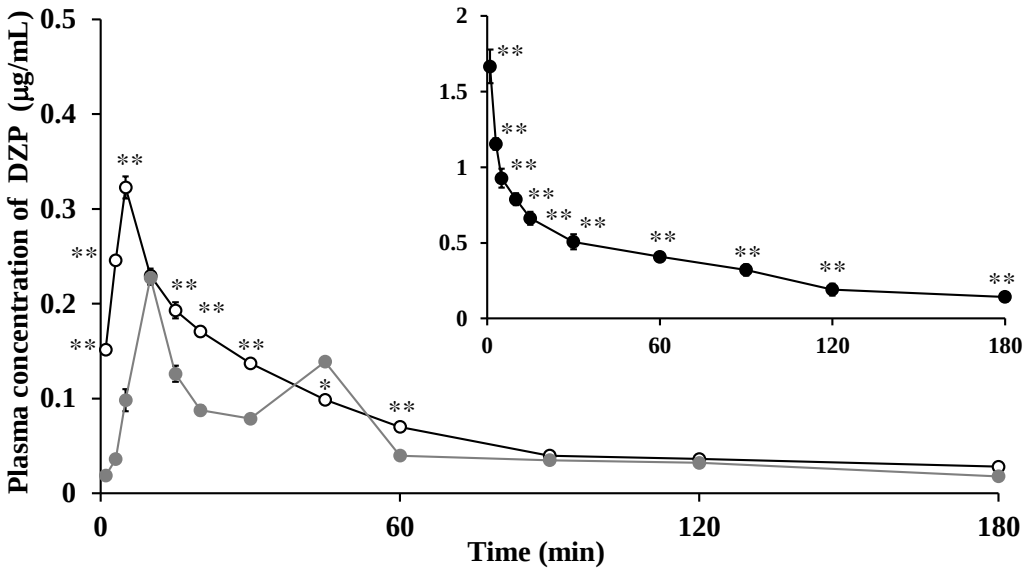

Figure 1: Plasma concentration profiles after *i.v.* (0.5 mg/kg), *i.n.* (0.5 mg/kg), and *i.r.* administrations (1.0 mg/kg) of DZP.

● : *i.v.* administration, ○ : *i.n.* administration, ● : *i.r.* administration.
Each data denotes the mean S.E. (n = 3–4).
* : $p < 0.05$, ** : $p < 0.01$ compared with *i.r.* administration in plasma concentration of DZP (student's t-test).

Table 2: Pharmacokinetic parameters of DZP following *i.v.* (0.5 mg/kg), *i.n.* (0.5 mg/kg), and *i.r.* administrations (1.0 mg/kg).

Routes	T_{max} (min)	C_{max} (μg/mL)	$AUC_{0\rightarrow180}$ (μg/mL·min)	$F_{0\rightarrow180}$
i.v.	-	-	65.7 ** ± 6.8	-
i.n.	4.5 ** ± 0.5	0.323 ** ± 0.016	13.7 ** ± 0.7	20.8 ** ± 1.0
i.r.	10.0 ± 0.0	0.227 ± 0.007	9.9 ± 0.6	7.5 ± 0.4

Each data represents the mean S.E. (n = 3–4).
** : $p < 0.01$ compared with *i.r.* administration (student's t-test).

3.1.2 Plasma and brain concentrations of DZP at 5 minutes after *i.v.*, *i.n.,* and *i.r.* administrations

Figure 2 shows DZP concentration in plasma, FB, and HB at 5 min after *i.v.*, *i.n.,* and *i.r.* administrations. At this time point, the rank order of DZP concentration was *i.v.* > *i.n.* > *i.r.* in all plasma and brain tissues (Figs. 2). DZP concentration in FB and HB after *i.n.* administration were 6.0- and 11.8-fold higher than those after *i.r.* administration, respectively, although plasma concentration after *i.n.* the administration was only 2.3-fold higher than that after *i.r.* administration (Fig. 2b and c). In comparison between *i.v.* and *i.n.* administrations, on the other hand, DZP concentration in FB and HB after *i.v.* administration was only 2.8- and 1.9-fold higher than those after *i.n.* administration (Figs. 2b and c). These concentration differences were comparable to that obtained in plasma (3.7-fold). To compare drug transfer to each brain tissue by different administration routes, the DZP concentration ratio in FB and HB against plasma concentration was calculated using the concentration data at 5 min (Fig. 3). The rank order of the concentration ratio was *i.n.* > *i.v.* > *i.r.* in both FB and HB, unlike the rank order of *i.v.* > *i.n.* > *i.r.* obtained with just concentration in Figure 2. The DZP concentration ratio in FB and HB after *i.n.* administration was significantly greater than those after *i.v.* and *i.r.* administrations (Fig. 3). These results suggest that brain delivery of DZP at 5 min after *i.n.* the administration is higher than that after *i.v.* and *i.r.* administrations.

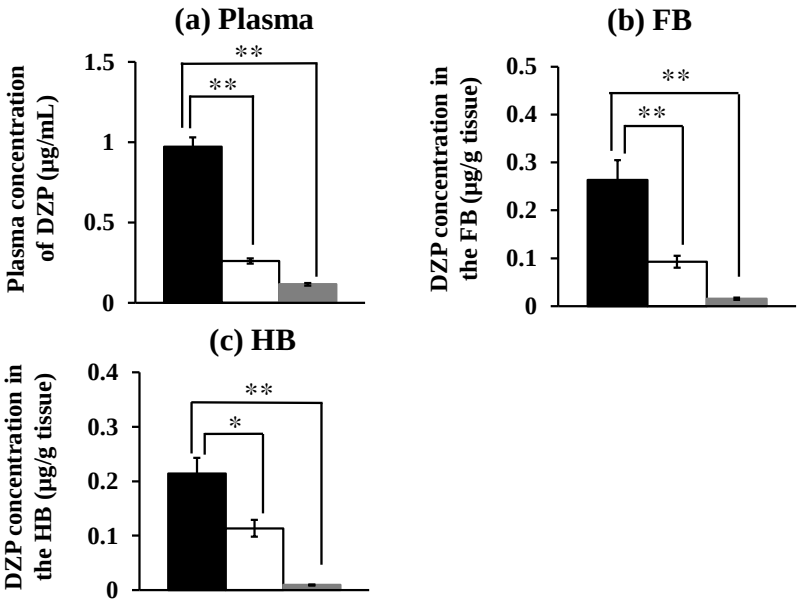

Figure 2: DZP concentration in (a) plasma and in the (b) FB and (c) HB at 5 min after *i.v.* (0.5 mg/kg), *i.n.* (0.5 mg/kg), *and i.r.* administrations (1.0 mg/kg).
■ : *i.v.* administration, □ : *i.n.* administration, ■ : *i.r.* administration.
Each data denotes the mean S.E. (n = 4-12).
* : $p < 0.05$, ** : $p < 0.01$ (Tukey-Kramer test).

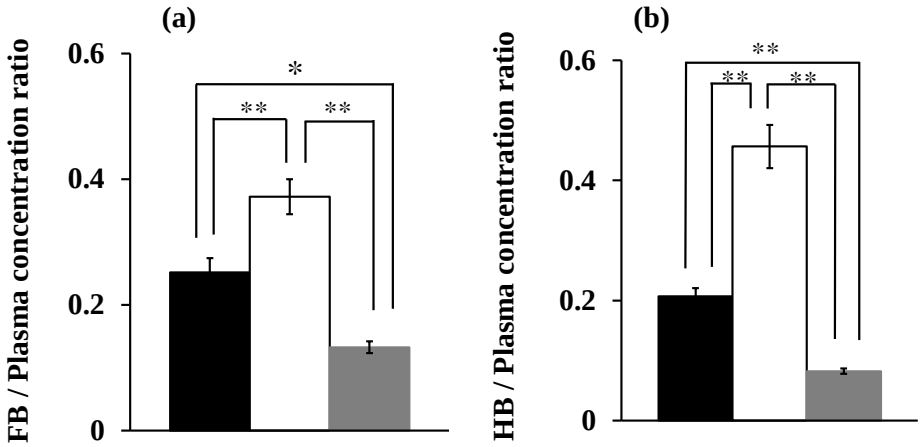

Figure 3: Ratio of DZP concentration in the (a) FB and (b) HB to DZP concentration in Plasma after *i.v.* (0.5 mg/kg) , *i.n.* (0.5 mg/kg), and *i.r.* administration (1.0 mg/kg) 5 min.
■ : ***i.v.* administration,** □ : ***i.n.* administration,** ■ : ***i.r.* administration.**
Each data denotes the mean S.E. (n = 4-12).
* : $p < 0.05$, ** : $p < 0.01$ (Tukey-Kramer test).

3.2 Seizure suppression effect of DZP

3.2.1 Seizure suppression effect of DZP after *i.v.*, *i.n.,* and *i.r.* administrations

Table 3 shows the seizure suppression rate and the seizure suppression time after DZP administration at a dose of 0.5 mg/kg (*i.v.* and *i.n.*) or 1.0 mg/kg (*i.r.*) in the PTZ-induced SE model rats. The seizure suppression rates after *i.v., i.n.,* and *i.r.* administrations were 100% (10/10), 80% (8/10), and 60% (6/10), and the seizure suppression times were 2.2 ± 0.5 min, 5.0 ± 0.3 min and 15.6 ± 1.7 min, respectively. The *i.n.* administration was more potent for seizure suppression compared with *i.r.* administration, while lesser than *i.v.* administration although significant differences were not observed (Table 3).

Table 3: Seizure suppression effect of DZP after *i.v.* (0.5 mg/kg) , *i.n.* (0.5 mg/kg), and *i.r.* administrations (1.0 mg/kg).

Routes	Seizure suppression rate (%)	Seizure suppression time (min)	
i.v.	100 (10/10)	2.2 ± 0.5	**
i.n.	80 (8/10)	5.0 ± 0.3	**
i.r.	60 (6/10)	15.6 ± 1.7	

Each data represents the mean S.E. (n=10). ** : $p < 0.01$ (Tukey-Kramer test).

3.2.2 Relationship between the anticonvulsant effect of DZP and plasma concentration immediately after seizure suppression

The seizure suppression time was plotted against the plasma DZP concentration obtained immediately after the seizure suppression with different administration routes and various administration doses (Fig. 4). Taken into the systemic circulation are thought to distribute to the brain parenchyma through the BBB. In correlation analysis targets at their three administration routes, a good correlation (r = −0.997, p = 0.00001) shown by the solid line in Figure 4 was obtained between the plasma DZP concentration and seizure suppression effect. On the other hand, data obtained from *i.n.* administration (2.0, 1.0, and 0.5 mg/kg) were plotted toward lower concentration and shortened seizure suppression time from the regression line for the other three administration routes (Fig. 4). These results indicate that the onset time of seizure suppression effect after *i.n.* administration is much earlier than the time predicted by the plasma DZP concentration and potential direct delivery of DZP from the nasal cavity to the brain may be involved in such rapid onset.

3.3 Analysis of DZP delivery route from nasal cavity to brain

Figure 5 shows DZP concentration profiles in the FB, HB, OB, TN, and CSF up to 60 min after *i.v.* and *i.n.* administrations, and obtained C_{max} and T_{max} in various brain tissues and CSF are shown in Table 5. T_{max} in FB and HB after *i.n.* and *i.v.* administrations were 3 min (Fig. 5 and Table 4). DZP concentrations in the OB at 3 min after *i.n.* administration was 1.2-fold greater than those after *i.v.* treatment, further 4.6- and 1.8-fold greater in the TN and CSF, respectively (Fig. 5 and Table 4). Notably, T_{max} in the TN and CSF after *i.n.* administration was shortened compared with that after *i.v.* administration. DTE and DTP, indicators of drug targeting efficiency in brain tissue and direct delivery from the nasal cavity to brain tissue, were calculated to estimate the possible involvement of the NTB route for direct delivery of DZP from the nasal cavity to the brain (Table 4). DTP values after *i.n.* administration was 95.9% for OB, 77.5% for CSF, and 97.2% for TN (Table 4). These results suggest that contribution of direct delivery to the brain via the NTB route can be dominant after *i.n.* administration of DZP, especially in the early stage up to 5 min after administration, compared with that of the indirect route through BBB from systemic circulation.

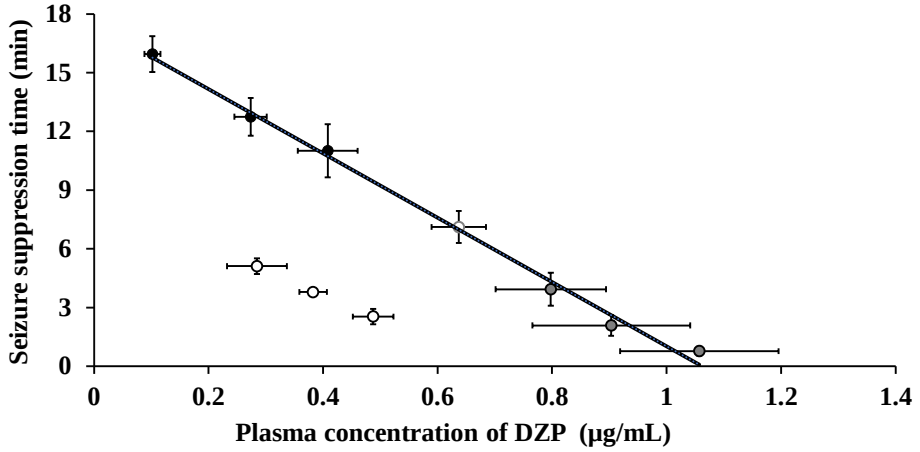

Figure 4: Relationship seizure suppression time after *i.v.* (1.0 mg/mL, 0.5 mg/mL, 0.2 mg/kg), *s.c.* (2.0 mg/kg), *i.n.* (2.0 mg/kg, 1.0 mg/kg, 0.5 mg/kg), and *i.r.* administration (4.0 mg/kg, 2.0 mg/kg, 1.0 mg/kg) in SE model rats and plasma concentration of DZP immediately after seizure suppression.

● : *i.v.* administration, ○ : *s.c.* administration, ● : *i.r.* administration, Solid line y = −16.427x + 17.445 (r^2 = 0.9946), r = −0.9973, p value = 0.00001.

○ : *i.n.* administration

Each data denotes the mean S.E. (n = 4–12).

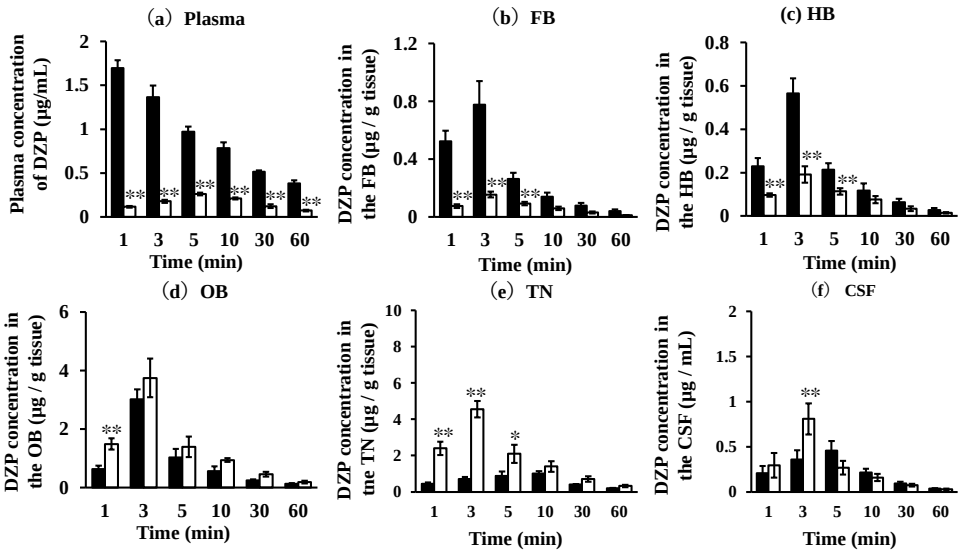

Figure 5: DZP concentration in (a) plasma and in the (b) FB, (c) HB, (d) OB, (e) TN, and (f) CSF after *i.v.* and *i.n.* administrations (0.5 mg/kg).
■ : *i.v.* administration, □ : *i.n.* administration.
Each data denotes the mean S.E. (n = 3).
* : $p < 0.05$, ** : $p < 0.01$ compared with *i.v.* administration (student's t-test).

Table 4: Pharmacokinetic parameters of DZP in the brain following *i.v.* and *i.n.* administrations (0.5 mg/kg).

	Routes	C_{max} (μg / g tissue)	T_{max} (min)	DTE	DTP (%)
Forebrain	*i.v.*	0.777±0.134	3.0 ± 0.0	-	-
	i.n.	0.155±0.352 **	3.0 ± 0.0	1.50	33.5
Hindbrain	*i.v.*	0.565±0.069	3.0 ± 0.0	-	-
	i.n.	0.192±0.038 **	3.0 ± 0.0	2.39	44.0
Olfactory bulb	*i.v.*	3.02±0.34	3.0 ± 0.0	-	-
	i.n.	3.75±0.658	3.0 ± 0.0	7.21	95.9
Trigeminal nerve	*i.v.*	1.00±0.14	10.0 ± 0.0	-	-
	i.n.	4.55±0.46 **	3.0 ± 0.0	8.89	97.2
CSF	*i.v.*	0.458±0.108	5.0 ± 0.0	-	-
	i.n.	0.809±0.172 *	3.0 ± 0.0	4.05	77.5

Each data denotes the mean S.E. (n = 3–9). *: $P < 0.05$, **: $p < 0.01$ compared with *i.v.* administration (student's t-test).

3.4 *Ex vivo* fluorescence imaging analysis using the lipophilic fluorescent substance RBB

Figure 6 shows fluorescence images of RBB in each brain tissue after *i.v. and i.n.* administrations of RBB. At 1 min after administration, a fluorescence signal was detected at the HB near the thalamus for *i.n.* administration (Fig. 6Ai), while at a portion of the FB for *i.v.* administration (Fig. 6Bi). Fluorescence signal after *i.v.* administration spreads from FB to HB within 3 to 10 min (Fig. 6A), and the strongest signal was detected in the FB area at 3 min (Fig. 6Aii). In the case of *i.n.* administration, fluorescent signal spreads from the HB to the FB within 3 to 5 min (Fig. 6B), and the strongest signal was detected at HB near the thalamus at 3 min (Fig. 6Bii). These results indicate that the distribution behavior of DZP in the brain after *i.n.* administration differs from that after *i.v.* administration. Focusing the NTB route, a fluorescence signal

was detected at TN and CSF at 1 min after *i.n.* administration (Fig. 6Bi), and in OB at 3 min (Fig. 6Bi). These signal intensities were maximized at 3 min, then decreased gradually (Fig. 6B). In the case of *i.v.* administration, the weak signal was detected at OB, CSF, and TN at 3, 5, and 10 min, respectively (Figs. 6 Aii-Aiv).

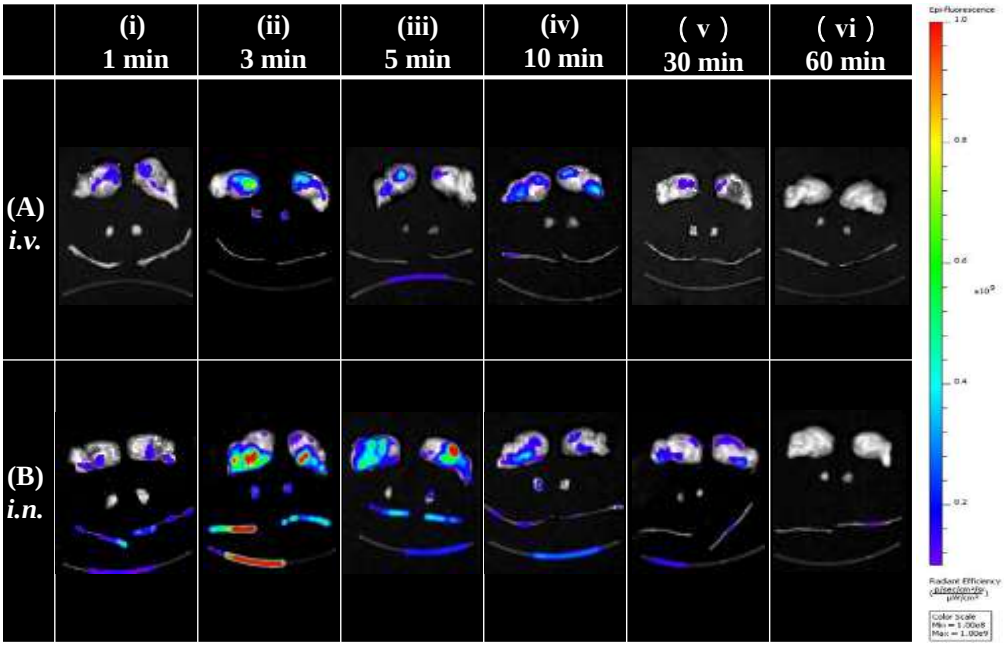

Figure 6: *Ex vivo* fluorescence imaging images in brain, OB, TN, CSF, after *i.v. and i.n.* administrations (0.5 mg/kg) of RBB. (n = 3)

4. DISCUSSION

In the present study, we evaluated the blood and brain kinetics and seizure suppression effect of DZP when DZP was administered into the nasal cavity allowed rapid onset for seizure suppression by direct distribution of DZP to brain tissues via NTB route in addition to indirect distribution from the systemic circulation via BBB. Based on pharmacokinetic data shown in Table 2, higher C_{max} and shortened T_{max} obtained by *i.n.* administration showed that DZP in the nasal cavity was absorbed into systemic circulation quickly compared with *i.r.* administration, although the extent of DZP absorbed was comparable between *i.n.* and *i.r.* administrations as indicated by comparable $AUC_{0\text{-}180}$ values. The C_{max} and T_{max} in plasma are the parameters expressing the rate of absorption, whereas the *AUC* reflects the extent of absorption. These findings suggest that *i.n.* administration offers better pharmacokinetic behavior for DZP in terms of the extent and the rate of bioavailability compared with the *i.r.* administration. In general, the brain concentration of a drug is proportional to plasma concentration if a drug distribution is following simple diffusion via BBB. Therefore, it is thought that the rank order of brain concentrations after *i.v.*, *i.n.,* and *i.r.* admnistrations coincide with that of plasma concentrations and their brain-to-plasma concentration ratios show approximate values. Indeed, their rank order of brain and plasma concentrations showed coincidence as shown in Figure 2, and the ratios after *i.v.* and *i.r.* administrations were approximately the same value between 0.1 and 0.2 (Fig. 3). On the other hand, the ratio after *i.n.* administration significantly increased compared with *i.v.* and *i.r.* administrations (Fig. 3), suggesting the possible involvement of the NTB route beside the distribution from plasma via BBB. As a result, the relationship between seizure suppression time and plasma concentration of DZP was examined to verify the pharmacokinetics and pharmacological effect of DZP. Generally, in *i.v.*, *s.c.,* and *i.r.*

dosing conducted in our study, drugs administered transfer into the brain via BBB from systemic circulation. As shown in Figure 4, the seizure suppression time after *i.v., s.c.,* and *i.r.* administrations of DZP was correlated well with plasma concentration. Kaurr et al. reported in their kinetic studies after *i.n.* administration of DZP in rabbits and rats where DZP was delivered to the brain via BBB [18]. If brain distribution of DZP after *i.n.* administration is explained by only delivery from the systemic circulation, the relationship between seizure suppression time and the plasma concentration can be plotted on the regression line in Figure 4 which is obtained from *i.v.*, *s.c.*, and *i.r.* administarations. However, data after *i.n.* administration was plotted to the short-time and low-concentration sides from the regression line, denoting that *i.n.* administration of DZP could provide a higher seizure suppression effect despite the low plasma DZP concentration. Findings obtained from pharmacological and pharmacokinetic studies strongly suggest that the direct delivery of DZP from the nasal cavity to the braine involves in *i.n.* administration. *I.n.* administered insulin, eletriptan, and caffeine are reported to deliver directly to the brain via the OB, TN, and CSF, in addition to pathways delivering them from the systemic circulation to the brain via the BBB [10-12]. Considering the possible involvement of direct transport through the NTB route, it is essential to assess DZP concentration profiles in the OB, TN, and CSF. The T_{max} of 3 min was obtained in FB, HB, OB, TN, and CSF after *i.n.* administration was shorter than that obtained in plasma (Fig. 5). T_{max} in each brain tissue and CSF were comparable or shorter than those after *i.v.* administration. DTE values calculated for OB, TN, and CSF in addition to FB and HB after *i.n.* administration of DZP was over 1 (Table 4), suggesting the possible involvement of a direct delivery route from the nasal cavity. Furthermore, the contribution of the NTB route to the brain delivery of DZP might be greater than that from the systemic circulation, since DTP for all brain tissues showed large values (Table 4). Using RBB having similar lipophilicity with DZP, *ex vivo* fluorescence imaging analysis in each brain tissue after *i.n.* administration was conducted. In fluorescence images from 1 to 3 min after *i.n.* administration, a strong fluorescence signal was detected in TN and brain region conceivably connecting to TN terminal (Figs. 6Bi and 6Bii). Furthermore, the fluorescence intensity in the TN at 1 and 3 min was strongest among the OB, TN, and CSF. These results provide that lipophilic compounds such as RBB as well as DZP are rapidly delivered to the brain tissues via the TN after *i.n.* administration and the TN might contribute more greatly to brain delivery than OB and CSF. The TN which innervates the respiratory epithelium in the nasal cavity enters the trigeminal ganglion, where a first neuron projects to the principal sensory trigeminal nucleus, and from there a second neuron projects to the thalamus via the trigeminal lemniscus [22]. Akita et al. revealed that GLP-2 derivatives appeared in the principal sensory trigeminal nucleus at 3 min after *i.n.* administration, then the derivatives were detected in the trigeminal ganglion and the trigeminal lemniscus at 5 min [22]. Furthermore, they described that GLP-2 derivatives detected in the TN fibers might be taken up by the cells in the TN and transferred to the principal sensory trigeminal nucleus [14]. It is of particular importance that antiepileptic drugs act on the thalamus and around the area to suppress the seizure in PTZ-induced SE animals [23]. Considering the similarity of lipophilicity of DZP and RBB, DZP administered into the nasal cavity is likely to be transferred to the adjacent thalamus through the TN pathway for developing the rapid seizure suppression effect, since TN reaches the thalamus via the principal sensory trigeminal nucleus to the trigeminal lemniscus. On the other hand, Kaurr et al. did not provide any findings for direct delivery from the nasal cavity to the brain as indicated by DTE and DTP values of 0.99 and 0.48, respectively [18]. As a result, T_{max} in various brain tissues after *i.n.* administration was observed at 3 min, and the concentrations in the OB, TN, and CSF at 1 and 3 min were higher than those of *i.v.* administration. Our data immediately after *i.n.* administration is essential to provide useful information on rapid onset of seizure suppression effect in the early stage of drug distribution after *i.n.* administration of DZP.

5. CONCLUSION

The present study elucidated an involvement of the direct delivery from the nasal cavity to the brain by the addition of

plasma and brain pharmacokinetics in the early stage after *i.n.* administration of DZP. The direct brain delivery of DZP was served by not only the olfactory nerve pathway known commonly as the main direct route but also the TN and CSF route. Particularly, DZP delivery to the vicinity of the thalamus via the TN route might be involved in the rapid onset of seizure suppression.

An adaptive drug for SE seizure suppression has other drugs except for DZP, which drugs possess different physicochemical properties. In the future, the establishment of the relationship of the physicochemical characteristic of drugs and the detailed property of distribution to the brain via NTB pathway when *i.n.* administration enables the drug selection and formulation preparation suitable for *i.n.* administration. We believe that the findings can lead to proposing an effective transnasal treatment targeted for the rapid onset of pharmacological action in the pivotal region.

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