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Pharmacokinetics and brain uptake of diazepam after intravenous and intranasal administration in rats and rabbits

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ABSTRACT

The purpose of this study was to investigate the plasma pharmacokinetics and brain uptake of a lipophilic benzodiazepine anticonvulsant, diazepam in New Zealand white rabbits and Sprague–Dawley rats to evaluate the possible absorption pathways after intravenous and intranasal administration. The intranasal formulation was prepared by dissolving DZ and 1% sodium glycocholate into microemulsion system composed of 15% ethyl laurate, 25% Labrasol®, 37.5% Transcutol®P, 12.5% ethanol, and 10% water. Diazepam was administered intravenously (1 mg/kg) or intranasally (2 mg/kg) to rats and rabbits. Drug concentrations in the plasma and six different regions of the brain tissues, i.e., olfactory bulb, olfactory tract, anterior, middle, and posterior segments of cerebrum and cerebellum were analyzed by LC/MS method after solid phase extraction. After IN administration, DZ was rapidly absorbed into the systemic circulation, and readily and homogenously distributed into the different regions of brain tissues with a t_{max} of 5 and 10 min in rats and rabbits, respectively. The bioavailability of DZ in rat plasma (68.4%) and brain (67.7%) were 32–47% higher than those observed in rabbit plasma (51.6%) and brain (45.9%). The AUC_{brain}/AUC_{plasma} ratios in rabbits after IN administration (3.77 ± 0.17) were slightly lower than from IV administration (4.23 ± 0.08). However, in rats the AUC_{brain}/AUC_{plasma} ratios after IV (3.03 ± 0.07) and IN (3.00 ± 0.32) administration were nearly identical. The plasma pharmacokinetic and distribution studies in the two animal models clearly showed that lipophilic DZ molecules reached the brain predominantly from the blood by crossing the blood–brain barrier after IN administration with no significant direct nose-to-brain transport via olfactory epithelium.

1. Introduction

Status epilepticus is a serious neurological emergency that affects almost 3 million people per year world wide (Treiman and Walker, 2006). The management of status epilepticus requires the rapid attainment of therapeutically relevant plasma and brain concentrations of antiseizure medications to minimize the risk of mortality, morbidity and permanent brain damage associated with prolonged seizure activity (McNamara, 1996). This necessitates the administration of lipophilic drugs that cross blood–brain barrier readily to achieve brain concentrations of the drug responsible for an acute antiepileptic effect (Kilpatrick, 1999). The lipophilic benzodiazepine derivative, diazepam (DZ) is a drug of choice for the first-line management of status epilepticus. Currently, diazepam is administrated via parenteral or rectal route for the rapid suppression of seizures. The disadvantages associated with intravenous administration include need for sterile equipment, skilled personnel, hypotension and cardiorespiratory depression (Lott, 1990). However, the rectal administration is inconvenient especially for adults under emergency situations. The nasal delivery of the diazepam has been reported as a feasible alternative to intravenous and rectal administration for the acute treatment of status epilepticus (Bechgaard et al., 1997; Li et al., 2000, 2002). In the development of nasal formulations, the low aqueous solubility of diazepam (less than 50 μg/mL) necessitates the use of non-irritating good solubilizing vehicles that are able to deliver an IV dose of 5–10 mg in a volume not exceeding 150 μL per nostril. The target solubility of the drug was estimated to be 33–67 mg/mL on the basis of the therapeutic IV dose (5–10 mg), effective nasal delivery volume (≤300 μL) and pharmacokinetic data (F = 50–75%) available thus far.

The advantages of nasal route for systemic delivery of diazepam can include rapid onset of action and the preferential drug delivery to brain via olfactory pathway (Illum, 2000). A number of hydrophilic and lipophilic therapeutic agents have been shown to enter the brain directly from the nasal cavity via olfactory pathway. Einer-Jensen and Larsen (2000) has reported that a preferential first pass distribution of diazepam to the rat brain may occur after nasal

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administration due to local transfer of drug between nasal venous blood and brain arterial blood in the cavernous sinus–carotid artery complex. However, their conclusions were based on the measurement of "Head" plasma/"Heart" plasma ratios after intravenous and intranasal administration of radioactively labeled diazepam. In our view, to elucidate the involvement of direct nose-to-brain pathway, the actual concentrations of drug should be simultaneously measured into the plasma and brain tissue.

As part of development studies of DZ intranasal delivery system for use in the acute treatment of epileptic seizure, the objective of the present study was to investigate the plasma pharmacokinetics and brain distribution profiles of a lipophilic anticonvulsant benzodiazepine, DZ, after IV and IN administration in two animal models, i.e., New Zealand white rabbits and Sprague–Dawley rats to assess whether there is the direct nose-to-brain transport pathway for the drug molecules. The additional aim of the present study was to evaluate the interspecies difference in the pharmacokinetics and brain distribution profiles of DZ after IV and IN dosing of same formulations to the two animal models, since only very few absorption studies, where the same IN formulations were administered to different animals, are available in the literature.

2. Materials and methods

2.1. Materials

Diazepam (DZ) was purchased from Sigma (St. Louis, MO, USA). D5-diazepam (1 mg/mL) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Ethyl laurate, ethanol, formic acid, sodium glycocholate, and EDTA were purchased from Sigma (St. Louis, MO, USA). Sodium pentobarbital (324 mg/mL) was obtained from Henry Schein (Melville, NY). Caprylcaproyl macrogol-8 glyceride (Labrasol®) and diethylene glycol monoethyl ether (Transcutol®P) were a kind gift from Gattefosse (Gattefosse, USA). All other chemicals were of high performance liquid chromatography or analytical grade and used as received. Deionized and distilled water was used.

2.2. Preparation of DZ test formulations

For IV administration, DZ injection (5 mg/mL) was prepared under aseptic conditions using the following composition: 40% propylene glycol, 10% ethanol, 5% sodium benzoate/benzoic acid, 1.5% benzyl alcohol and q.s. with water. The intranasal microemulsion formulation was selected from a ternary phase diagram of oil (ethyl laurate), surfactant (Labrasol®)/cosurfactant (Transcutol®P and EtOH) constructed using water titration method at ambient temperature to obtain concentration range of components that can result in microemulsion region. Surfactant was mixed with the cosurfactant mixture in a fixed weight ratio and the resultant mixture was named as S_{mix}. To draw the phase diagram, the ratio of oil to S_{mix} was varied from 9:1 (w/w) to 1:9 (w/w). The water was added drop-wise to each oil–S_{mix} mixtures with constant stirring. After equilibrium, the samples were visually observed for phase clarity, flowability, and identified as microemulsion regions. After identification of microemulsion region in the phase diagram, the microemulsion formulation was selected at the desired component ratios from Fig. 1. The physical stability was studied via visual observation of clarity, phase separation, and particle size determination at room temperature up to 2 months. The droplet size analysis of selected microemulsion was conducted using a dynamic light scattering method with a Nicomp 380-Submicron Particle Sizer (Particle Sizing Systems, Santa Barbara, CA).

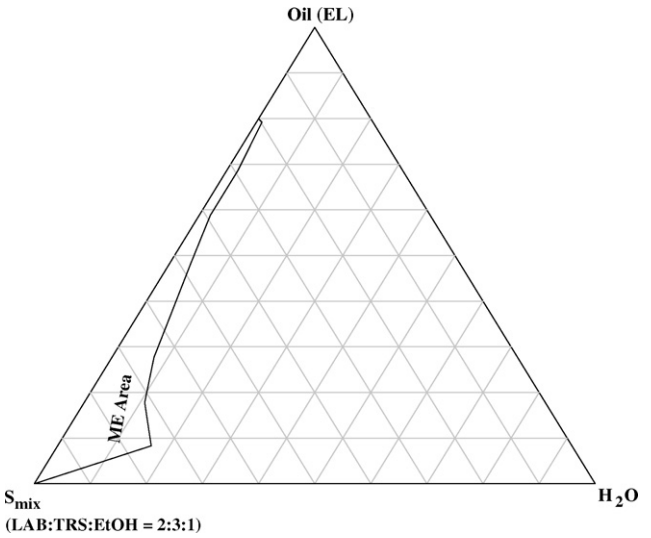

Fig. 1. Phase diagram of oil (EL)-surfactant/cosurfactant mixture (LAB:TRS:EtOH ratio of 2:3:1) and water system.

2.3. In vivo pharmacokinetic and brain distribution studies

2.3.1. Animals

New Zealand white rabbits (2.5–3.5 kg), obtained from Marland Breeding Farm Inc. (Hewitt, NJ), and Sprague–Dawley rats (350–400 g), obtained from Taconic Farms (Germantown, NY), were used in the pharmacokinetic studies. All experiments were conducted according to the protocol for animal use approved by the Animal Research Ethics Committee (IACUC) at St. John's University. Animals were housed in individual cages with free access to food and water in a room with an automatically controlled illumination (a 12 h light–dark cycle), temperature and relative humidity.

2.3.2. Comparative nasal absorption studies in rabbits

Conscious New Zealand white rabbits (2.5–3.5 kg) were used in these plasma PK experiments with a wash out period of 7 days. Prior to drug administration, the rabbits were weighed and restrained in the restrainer. For comparative PK studies with IV and IN formulations, a cross over design with 3 rabbits for each formulation was used. For IN administration, 3 and 6% DZ microemulsion formulations were examined. The rabbits received an IV dose of 1 mg/kg via marginal ear vein, whereas IN dose was administered to the rabbits at 1 and 2 mg/kg to test the dose–progression effect. For IN administration, each rabbit received 50 μL of IN formulation into each nostril within 10 s by means of Pfeiffer spray device (Pfeiffer, Princeton, NJ). Blood samples (1 mL) were collected at 0 (pre-dosing), 2, 5, 10, 20, 40, 60, 120, 180 and 240 min after IV and IN administration via an artery catheter set up at rabbit ear. All blood samples were collected into the test tubes containing anticoagulant EDTA, and plasma samples were separated by centrifugation at 3120 × *g* for 15 min and stored at −40 °C until analysis using the LC/MS via solid phase extraction (SPE) method.

2.3.3. Nasal absorption and distribution studies in rabbits and rats

2.3.3.1. Rabbit study. Conscious male New Zealand white rabbits were used after division into two groups. One group (3 rabbits for each time point × 5 time points = 15 rabbits) received IV injection at 1 mg/kg dose and the other group received IN microemulsion at 2 mg/kg dose by spraying 50 μL of IN formulation into each nostril within 10 s using a metered-dose pump spray device (Pfeiffer, Princeton, NJ). At predetermined time intervals, i.e., 5, 10, 20, 40

and 60 min after dosing, the blood samples (3 mL) collected from rabbit ear artery into EDTA-treated test tubes were centrifuged for 15 min at 3120 × *g*.

Thereafter, the rabbits were euthanized with an overdose of pentobarbital (100 mg/kg) and CSF samples (1 mL) were withdrawn by the technique of cisternal puncture (Dahlin and Björk, 2000). Briefly, atlanto-occipital membrane was exposed and 1 mL of the CSF was withdrawn through the membrane by gentle suction using a 23-gauge 1 in. long needle attached to a disposable syringe. Upon finishing the CSF sampling, an incision was made in the skin over the skull of rabbits with a bone saw. Then the skull was cut open and the intact brain including olfactory bulb (OB), olfactory tract (OT), cerebrum (CB,) and cerebellum (CL) was carefully removed from the skull. The brain was quickly rinsed with normal saline and wiped with Kimberly-Clarks wipes to remove the stinted blood. After collection, all biological samples such as plasma, CSF and brain tissue were immediately stored at −40 °C until analysis. Approximately, 100 mg of various regions of the brain tissue was weighed for determination of DZ concentration in these samples.

2.3.3.2. Rat study. Fifty (50) male Sprague–Dawley rats weighing 350–400 g were randomly divided into two groups. Prior to experiment, the rats were anesthetized with isoflurane. For IV administration, one group (5 rats for each time point × 5 time points = 25 rats) was given IV injection by tail vein at 1 mg/kg dose. For IN administration, the animals were placed on one side and 20 μL (2 mg/kg) of the formulation was given via a PE 10 tube attached to a microliter syringe inserted into each nostril of rat about 10 mm. At predetermined time intervals, i.e., 5, 10, 20, 40 and 60 min after dosing, the blood samples (5 mL) were collected by cardiac puncture into EDTA containing test tubes and centrifuged at 3120 × *g* for 15 min to obtain plasma samples. Thereafter, the rats were euthanized by exposure to gaseous CO_2 and various regions of brain tissue were collected in a manner similar to that described for rabbits.

2.4. Analytical procedure

DZ in various biological samples was assayed using the LC/MS method. Oasis cartridges (Waters, Millford, MA, USA) were used for the extraction of diazepam from plasma and brain tissue samples. Firstly, the cartridges were preconditioned by flushing the cartridge with 1 mL of methanol followed by 1 mL of distilled water. Plasma samples (500 μL) spiked with different concentrations of DZ containing 100 ng/mL I.S. (D5-diazepam) were poured into the cartridges on a vacuum suction manifold (Supelco, Visiprep™ 24, Bellefonte, PA, USA). Endogenous impurities were removed by washing the cartridges with 2 mL of 90:10 water:methanol followed by 2 mL of 75:25 water:methanol basified with 2% ammonium hydroxide. Finally, DZ and D5-diazepam were eluted with 1.5 mL of 80:20 methanol:water containing 2% acetic acid and evaporated to dryness under vacuum. The resultant residue was reconstituted into 0.2 mL of mobile phase and injected into HPLC/MS.

To extract the DZ from the brain tissues, the brain slices (100 mg) obtained from various regions of the brain were homogenized using VirTis Tissue Homogenizer at 25,000 rpm for 1 min after addition of 1 mL of ethyl ether, a specified amount of DZ and internal standard to achieve a final concentration of 100 ng/mL of IS. After centrifugation of the brain homogenate–ethyl ether mixture at 3124 × *g* for 20 min at 10 °C in a refrigerated centrifuge, the supernatant was taken out and evaporated under vacuum to dryness. The residue was reconstituted with 1 mL of distilled water, vortexed briefly followed by sonication for 15 min to obtain dispersion. The prepared dispersion was then loaded onto preconditioned Oasis HLB cartridge and processed as for plasma.

The analytical system consisted of Shimadzu LC-10ADVP integrated HPLC system controlled by Analyst software 1.4 (Applied Biosystems, Toronto, Canada). Mass spectrometer PE Sciex API 150 EX coupled to HPLC column via a turbo-ionspray interface was operated in the positive ion scan mode under total ion chromatography (TIC) and extracted ion chromatography monitoring (XIC). The optimized MS parameters were: turbo gas 7 L/min, nebulizer gas 10, curtain gas 8, declustering potential 32.0 V, focusing potential 172.9 V, entrance potential 4.4 V and a temperature of 450 °C. The protonated molecules of diazepam and D5-diazepam were detected at an *m*/*z* ratio of 285.1 and 290 amu, respectively. Isocratic chromatographic separation was performed on a Narrow bore Atlantis dC_{18} column (100 mm × 2.1 mm I.D., 3 μm, Waters) equipped with a Atlantis dC_{18} guard column (10 mm × 2.1 mm I.D., 3 μm, Waters), using the mobile phase consisting of 0.05% formic acid in 75% methanol/25% water delivered to the MS at a flow rate of 180 μL/min. The injection volume was 10 μL. The retention time on an average was 5 min for both diazepam and D5-diazepam. The calibration curves prepared in the range of 5–500 ng/mL for plasma and 10–250 ng/mL in the brain samples were linear with correlation coefficients $r^2 > 0.9971$ ($n = 6$). The mean % recoveries of DZ from the plasma and brain tissue samples were 92.4 and 84.2%, respectively.

2.5. Data analysis

All concentration data were dose and weight normalized, and then analyzed using WinNolin software (Pharsight Corporation, Cary, NC). The peak plasma concentrations and the t_{max} values of nasal administration were measured directly from the plasma concentration–time data. The area under the plasma concentration–time curve was calculated by using the linear/logarithmic trapezoidal rule. The absolute bioavailability of DZ after nasal administration of microemulsion was calculated by using the following Eq. (1)

$$F(\%) = \frac{\mathrm{AUC_{IN}}}{\mathrm{AUC_{IV}}} \times \frac{\mathrm{Dose_{IV}}}{\mathrm{Dose_{IN}}} \times 100 \quad (1)$$

Based on the determined diazepam concentrations in the different regions of the brain and mean weight fraction of various regions of brain tissue such as olfactory bulb (OB), olfactory tract (OT), cerebrum (CB) and cerebellum (CL), the total amount of DZ (Q_b) and mean DZ concentration in the whole brain were calculated using the following Eqs. (2) and (3):

$$Q_b = (C_{OB} \times F_{OB} + C_{OT} \times F_{OT} + C_{CB} \times F_{CB} + C_{CL} \times F_{CL}) \times W_B = \sum (C_B F_B) \times W_B \quad (2)$$

where *C* represents drug concentration in various regions of brain tissue; *F* is percentage weight fraction of various brain tissues; and W_B is the whole brain weight.

$$C_{mean} = \frac{Q_b}{W_B} = \sum (C_B F_B) \quad (3)$$

The brain targeting efficiency after nasal administration was calculated using drug targeting efficiency index that represents a time–average distribution ratio of the drug in the brain to the drug in plasma as follows (Chow et al., 1999):

$$\mathrm{DTE} = \frac{\mathrm{AUC_{brain}}}{\mathrm{AUC_{plasma}}}$$

where AUC_{brain} was calculated from the mean drug concentration in the whole brain tissue versus time curve using the log/linear trapezoidal method without extrapolation to infinity.

3. Results and discussions

3.1. Microemulsion composition

The microemulsion formulation consisting of 15% ethyl laurate, 25% Labrasol®, 37.5% Transcutol®P, 12.5% ethanol, and 10% water was selected from the ME area of phase diagram (Fig. 1). The test microemulsion formulations were prepared by dissolving 1% sodium glycocholate, and 3% or 6% DZ into the microemulsion formulation. The microemulsion formulation provided high solubilization capacity of DZ (60 mg/mL) allowing the development of an IN formulation containing 18 mg/nasal spray (300 μL). The mean droplet size of the microemulsion was found to be 48.1 ± 4.5 nm. The microemulsion systems showed no significant change in particle size, clarity, and phase separation after storage at room temperature for a period of 2 months, indicating good physical stability. In addition, DZ microemulsion formulation was found to be chemically stable in the accelerated stability test program at 45 °C for 2 months.

3.2. Weight fraction of various regions of rabbit and rat brain

In order to determine the drug distribution pattern in the various regions of the brain, the weight fraction of various parts of rabbit and rat brain tissues, i.e., olfactory bulb (OB), olfactory tract (OT), cerebrum (CB) and cerebellum (CL) was needed. Due to lack of such information in the literature, the weight fraction of the OB, OT, CB and CL was determined using 12 rabbits and 20 rats. The mean weight percentages of the different regions of brain tissue are listed in Table 1. The whole brain weight was approximately 7–10 g for the rabbits weighing 2.5–3.0 kg, and about 1.75–1.95 g for rats with a body weight of about 350–400 g. Thus, average percent weight of brain was approximately 0.3 and 0.5% of total body weight, in rabbits and rats, respectively.

3.3. Nasal absorption of DZ

The time courses of the plasma levels of DZ following intravenous DZ injection (1 mg/kg) and the intranasal administration of selected microemulsion formulation in rabbits at two different doses 1 and 2 mg/kg are presented in Fig. 2. The corresponding non-compartmental PK parameters determined using WinNonlin software are summarized in Table 2. The PK results demonstrate that the IN administration of DZ microemulsion produced very fast absorption rate with the t_{max} ranging from 2 to 10 min depending on the dose. The bioavailability of the DZ from microemulsion was found to be 64.2–65.4% on the basis of the $AUC_{0-4\,h}$ values determined after IN administration of the microemulsion at 1 and 2 mg/kg doses. In the acute treatment of status epileptic seizures, the rapid onset of anticonvulsant is desired. For this purpose, the AUC area obtained in the time period of 0–40 and 0–60 min were also calculated for the in vivo evaluation. As shown in Table 2, when the dose level increased twice, the C_{max} and the AUC values determined for time intervals (0–40, 0–60 and 0–240 min) were nearly doubled suggesting that the intranasal absorption of DZ from the microemulsion formulation was dose-proportional over 1–2 mg/kg dosing level. The IN bioavailability (64.2%) was unaffected by the DZ dose. However, when the IN dose was doubled, the t_{max} was increased to 10 min from 2 min at 1 mg/kg dose as shown in Table 2. The results showed that the intranasal absorption rate was decreased with increasing dosing level. The results of in vivo absorption study indicate that double dose of IN administration of DZ in microemulsion formulation (2 mg/kg) dose provided a comparable plasma drug concentration–time profile with that of IV administration of a 1 mg/kg dose with a bioavailability of 64.2%. The $AUC_{0-40\,min}$ and $AUC_{0-60\,min}$ of ME at 2 mg/kg dose reached about 105 and 102% of IV administration at 1 mg/kg. Therefore, based on the results of dose–response pharmacokinetics, DZ loaded IN microemulsion at a dose of 2 mg/kg and IV injection at a dose of 1 mg/kg was selected to study the DZ distribution into various regions of rabbit brain tissue after IV and IN administration.

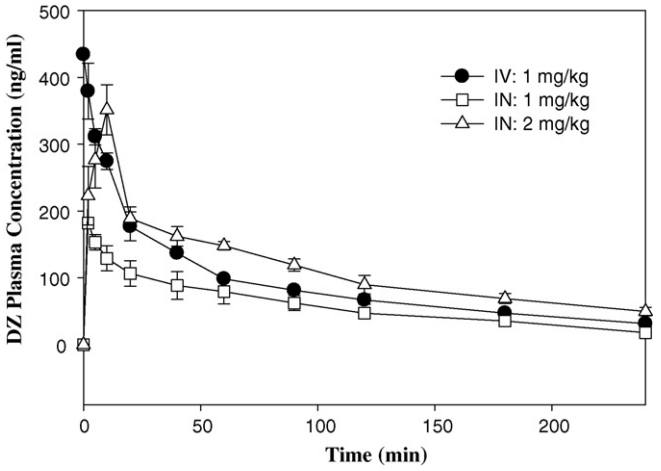

Fig. 2. Mean plasma concentration–time profiles of DZ after IV and IN administration of DZ microemulsion at 1 and 2 mg/kg doses in rabbits. Data represent the mean ± S.D. ($n = 3$).

3.4. In vivo absorption, CSF and brain distribution studies of DZ in rabbits

This study was undertaken to investigate whether or not DZ is transferred along the olfactory pathway to the CSF and brain following intranasal administration. In order to evaluate the effect of DZ microemulsion formulation on nasal systemic absorption of DZ, and its CSF and brain distribution, we simultaneously determined the levels of DZ in plasma, CSF and various regions of brain tissue, i.e., olfactory bulb (OB), olfactory tract (OT), anterior (CB1), middle (CB2) and posterior cerebrum (CB3) and cerebellum (CL) after IV and IN administration. Figs. 3–5 represent the DZ concentration–time profiles in the plasma, brain tissue and CSF determined after IV (1 mg/kg) and IN (2 mg/kg) administration of DZ formulations in rabbits. The pharmacokinetic parameters obtained from the simultaneous PK studies in rabbits are listed in Table 3. The bioavailability of the IN administration based on $AUC_{0-60\,min}$ was found to be 51.6% (Table 3). This bioavailability

Table 1
Weight fraction percentage of different regions of brain tissues of rabbits ($n = 12$) and rat ($n = 20$)

Animal model	Brain tissue	Olfactory bulb	Olfactory tract	Cerebrum	Cerebellum
Rats	Percentage (%)	4.05	5.63	76.40	13.92
	S.D.	0.41	0.57	1.92	2.15
Rabbits	Percentage (%)	2.68	4.67	72.36	20.29
	S.D.	0.52	0.78	3.57	3.47

Table 2
Bioavailability and pharmacokinetic parameters of DZ after IV and IN administration of microemulsion formulation to rabbits

Route/formulation	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (min)	$t_{1/2}$ (min)	$AUC_{0-40\ min}$ (h ng/mL)	$AUC_{0-60\ min}$ (h ng/mL)	$AUC_{0-240\ min}$ (h ng/mL)	F^a (%)
IV	0	416.6 ± 59.9	0	1.83 ± 0.19	139.4 ± 7.7	193.2 ± 29.5	355.1 ± 18.2	100.0
IN/ME	1	181.8 ± 3.7	2.0	1.49 ± 0.22	75.1 ± 11.0	103.0 ± 17.4	232.5 ± 31.5	65.4 ± 8.3
IN/ME	2	351.4 ± 37.5	10.0	1.91 ± 0.30	145.9 ± 12.6	197.5 ± 15.5	455.1 ± 17.5	64.2 ± 4.4

Data are presented as mean ± S.D. (n = 3). IN: intranasal. Dose-1 mg/kg: 30 mg/mL DZ in microemulsion. Dose-2 mg/kg: 60 mg/mL DZ in microemulsion.
[a] F (%) calculated based on AUC from 0 to 240 min. $C_{max,IV}$ was estimated by extrapolating the linear portion of initial plasma level data to y-axis.

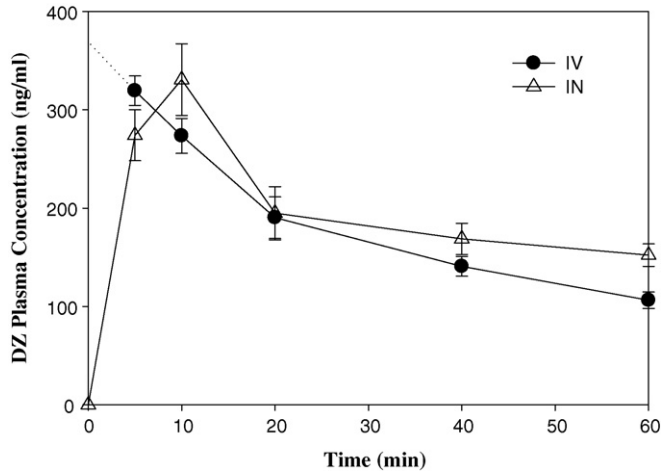

Fig. 3. Mean DZ concentration–time profiles in the plasma after IV (1 mg/kg) and IN administration of DZ microemulsion (2 mg/kg) in rabbits (n = 3 at each time point).

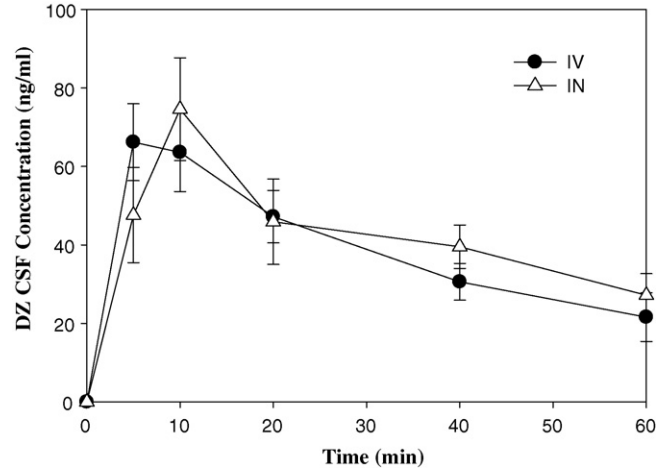

Fig. 4. Mean DZ concentration–time profiles in the CSF after IV (1 mg/kg) and IN administration of DZ microemulsion (2 mg/kg) in rabbits (n = 3 at each time point).

value appeared to be really comparable with the F value based on $AUC_{0-60\ min}$ (51.1–53.3%) found from the previous PK studies based on $AUC_{0-60\ min}$. As shown in Figs. 3–5 and Table 3, the IV and IN administration of the DZ formulations produced fairly comparable mean DZ concentration–time profiles in the plasma, CSF and brain tissues when the doubled dose of DZ was administered via intranasal route although there was a slight difference between $AUC_{plasma,IV}$ and $AUC_{plasma,IN,}$; $AUC_{CSF,IV}$ and $AUC_{CSF,IN}$ and between $AUC_{brain,IV}$ and $AUC_{brain,IN}$. The statistical analysis of these data (Student's t-test, two-tail) indicated that there was no significant difference between these two AUC values ($P > 0.05$). These results indicate that after nasal delivery, a lipophilic compound DZ ($\log P = 2.8$) is rapidly absorbed into the systemic circulation, from where the non-protein bound DZ probably enter readily into the CSF and brain tissues by crossing the blood–brain barrier as the major transport pathway. No extra direct nose-CSF/brain tissue transport could be demonstrated in rabbits. In the literature, intravenous infusion of a very lipophilic compound, progesterone ($\log P = 4.03$) showed comparable CSF concentration–time profiles when compared with the nasal delivery of the lipophilic steroid hormone (Van den Berg et al., 2004).

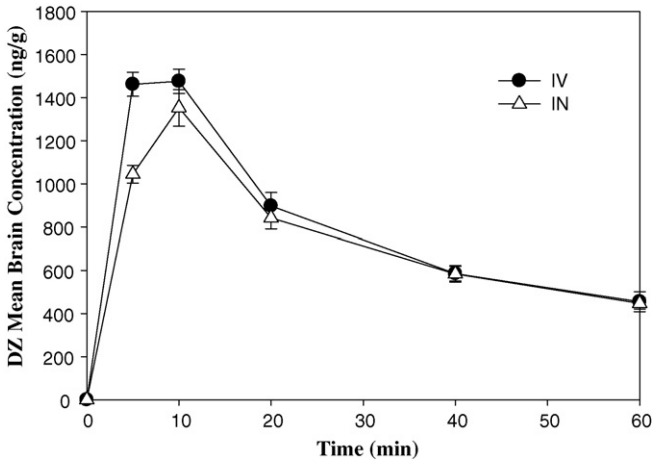

Fig. 5. Mean DZ concentration–time profiles in the brain tissue after IV (1 mg/kg) and IN administration of DZ microemulsion (2 mg/kg) in rabbits (n = 3 at each time point).

In the plasma and CSF, IV delivery brought about a faster onset of action with a t_{max} of 5 min than the IN dosing with a t_{max} of 10 min. However, in the brain distribution profile, the IV and IN administration showed identical t_{max} value of 10 min in the brain. The IV profile followed a concentration plateau around C_{max} at 5–10 min. These results are in agreement with the study conducted

Table 3
Pharmacokinetic parameters of DZ after IV and IN administration of microemulsion formulation to rabbits

Route	Dose (mg/kg)	C_{max} (ng/mL g)	t_{max} (min)	$AUC_{0-60\ min}$ (ng h/mL or g)	AUC_{IN}/AUC_{IV} (%)
Plasma–IV	1.0	373.1 ± 10.7	0	187.4 ± 13.8	100.0
Plasma–IN	2.0	330.7 ± 36.4	10	193.3 ± 19.9	51.6 ± 4.5
CSF–IV	1.0	66.2 ± 9.8	5	38.7 ± 6.3	100.0
CSF–IN	2.0	74.6 ± 13.1	10	42.1 ± 8.1	54.4 ± 16.5
Brain–IV	1.0	1475.8 ± 55.9	10	792.7 ± 44.2	100.0
Brain–IN	2.0	1352.7 ± 85.2	10	728.4 ± 42.9	45.9 ± 4.7

Data are presented as mean ± S.D. (n = 3).

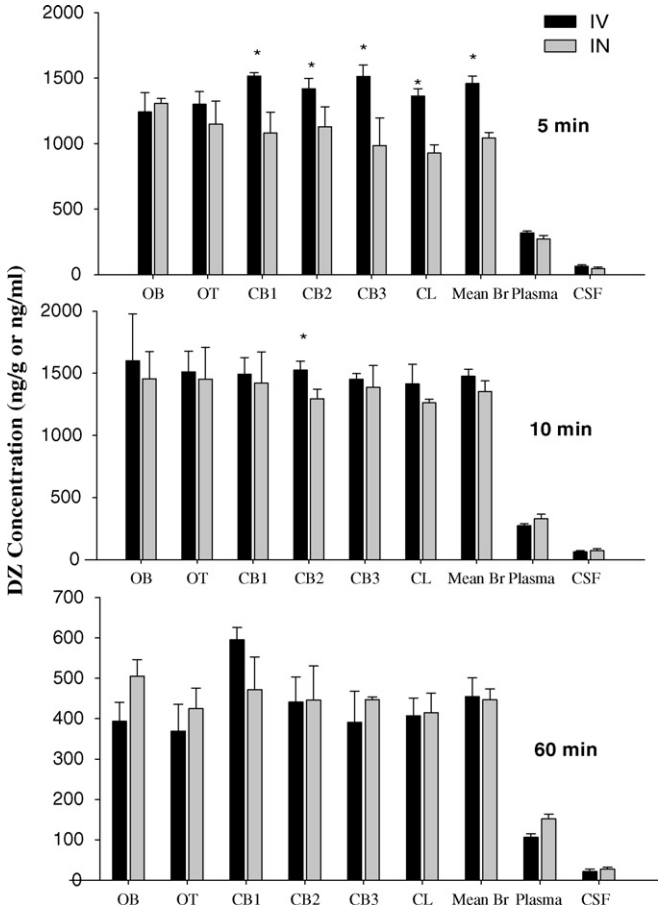

Fig. 6. DZ concentrations (ng/g or ng/mL) in various biological samples at 5, 10 and 60 min after IV (1 mg/kg) and IN (2 mg/kg) administration of DZ microemulsion in rabbits ($n = 3$ at each time point). $^{*}P < 0.05$.

by Tedeschi et al. (1983). These authors determined the rate of entrance of diazepam into the brain by recording the eye movement after IV administration of single dose of diazepam (5 mg) in six healthy male volunteers. The results of their study demonstrated that DZ crosses the blood–brain barrier very quickly and reaches its maximum pharmacological effect at approximately 10 min after IV administration; however, no kinetic data were available.

The mean C_{max} values in the brain (1352.7–1475.8 ng/g) were found to be markedly greater than those obtained in the plasma (330.7–373.1 ng/mL) and CSF (66.2–74.6 ng/mL). The observed fast systemic absorption and greater uptake of DZ in brain than CSF and plasma can be explained on the basis of its lipid solubility ($\log P = 2.8$). The distribution patterns of the DZ in various regions of the brain tissue such as OT, OB, CB and CL determined at 5, 10 and 60 min after IV and IN administration in rabbits are shown in Fig. 6. In these figures, the plasma and CSF drug level data are also included for comparison. The drug distribution results clearly indicate that the drug was homogeneously distributed into the various parts of the brain after both IV and IN administration although the IV administration (1 mg/kg) provided a significantly greater drug level noticed in the cerebrum than the drug level obtained at 5 min after IN administration (2 mg/kg). However, on the whole, the IV and IN administrations provided nearly homogeneous distribution of DZ into the various sections of the brain.

Table 4
Interspecies comparison of nasal cavity characteristics (Gizurarson, 1990; Meisami et al., 1990; Illum, 2000)

	Rats	Rabbits	Humans
Nasal length (cm)	2.3	5.2	7.5
Nasal volume (mL)	0.4	6.0	20.0
Nasal surface area (cm^2)	14.0	61.0	150.0
Olfactory region area (cm^2)	≅7.0	≅6.0	10.0
Clearance half-life (min)	5.0	10.0	15.0
CSF volume (mL)	0.15	2.3	100.0
CSF turnover rate (mL/h)	0.18	0.6	21.0

3.5. In vivo absorption and brain distribution studies of DZ in rats

The proper selection of animal models for studying the absorption and distribution of drugs across the nasal mucosa is of great importance when the results are to be extrapolated to man. The rat is one of the most widely used animals for drug delivery studies, in particular, for intranasal systems. The anatomic and physiological characteristics of the nasal cavity of rats, rabbits and man that may be influential to the absorption of drugs are summarized in Table 4. There has been a growing number in the utilization of the rabbits as an animal model for the in vivo absorption studies via a nasal route. To our knowledge, very few comparative absorption and distribution data are available in which the same test formulation is administered to different species. For comparison the rabbits have a significantly greater surface area of nasal mucosa (by 4.3 times) and nasal volume (by 15 times) than rats. In contrast, the rat model has a markedly greater percentage (≅50%) of olfactory region area in the nasal cavity when compared with that covered in the olfactory region of the rabbit nasal cavity. In addition, there is a noticeable difference in the mucociliary clearance rate between the rabbits and rats in which the latter show a 2 times faster clearance rate than the former. In order to evaluate these anatomical and physiological difference in the nasal cavity of two animal models, the PK and distribution studies in the plasma and brain tissue were additionally conducted in rats and the results of in vivo studies were compared with those corresponding data previously generated using rabbit as animal model.

The mean DZ concentration–time profiles in the plasma and brain determined after IV (1 mg/kg) and IN (2 mg/kg) administration of DZ microemulsion in rats are presented in Figs. 7 and 8, respectively. As shown in Table 5, the IN administration of DZ in microemulsion formulation at a 2 mg/kg dose resulted in a bioavail-

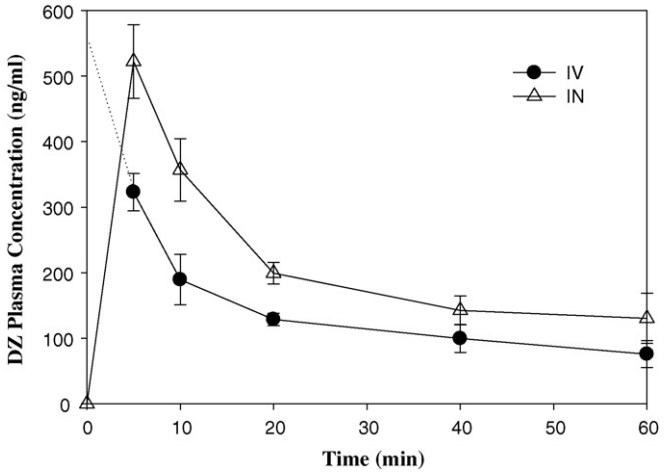

Fig. 7. Mean DZ concentration–time profiles in the plasma after IV (1 mg/kg) and IN administration of DZ microemulsion (2 mg/kg) in rats ($n = 5$ at each time point).

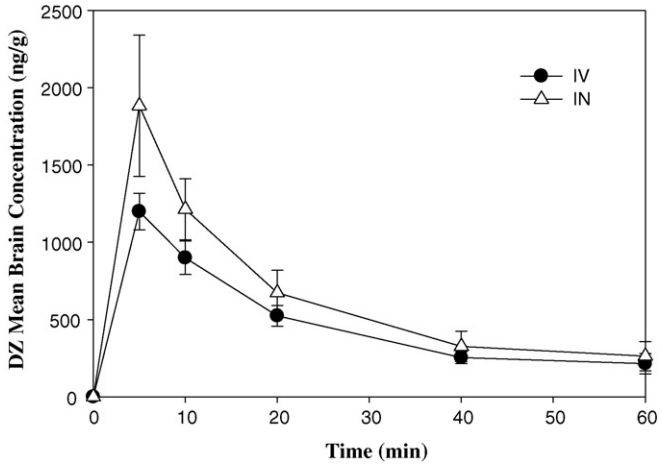

Fig. 8. Mean DZ concentration–time profiles in the brain tissue after IV (1 mg/kg) and IN administration of DZ microemulsion (2 mg/kg) in rats ($n = 5$ at each time point).

ability of 68.4 ± 14.1% based on the $AUC_{0-60\,min}$. The comparison of PK data obtained with two animal models (Tables 3 and 5) indicated that DZ intranasal bioavailability determined in the rats appeared to be 1.3 times greater than that obtained in the rabbits. However, IN delivery in rats brought a faster onset of action with a t_{max} value of 5 min as compared with rabbit model (t_{max} of 10 min). The interspecies differences in diazepam bioavailability have been also reported by Lindhardt et al. (2002). The t_{max} values of 5 and 10 min in rats and rabbits, respectively, indicate that DZ is rapidly absorbed into systemic circulation and readily distributed into the CSF and the brain tissues after IV and IN administration. The observed fast systemic absorption and rapid brain distribution process of diazepam can be explained on the basis of its lipophilic nature ($\log P = 2.8$). Similar to the results of brain distribution study in rabbits, homogenous distribution patterns of DZ were observed into the various regions of rat brain from 5 to 60 min after IV and IN administration (Fig. 9). However, in contrast to rabbit study, following IN administration of DZ microemulsion at a dose of 2 mg/kg, the drug levels in all regions of brain tissue were 1.2–1.8-fold higher than those of IV administration at 5, 10 and 20 min.

The results of simultaneous plasma PK and brain distribution studies revealed that the bioavailabilities of DZ in rat plasma (68.4%) and brain (67.7%) were 32–47% higher than those observed in rabbit plasma (51.6%) and brain (45.9%). Possible explanation for greater bioavailabilities obtained with rat model may be due to the following factors such as (1) application of anesthesia to animal, (2) animal position during nasal administration, and (3) mucosal area covered by formulation. For instance, application to anesthesia can decrease the nasal mucociliary clearance in rats, thereby increasing the residence time and nasal absorption of DZ from the nasal mucosa. In rats, lying on their side during drug administration by polyethylene tubing, it is likely that the formulation is covering a large area of nasal mucosa for a longer period of time resulting in greater nasal absorption. The 1.3-fold higher bioavailability in rats

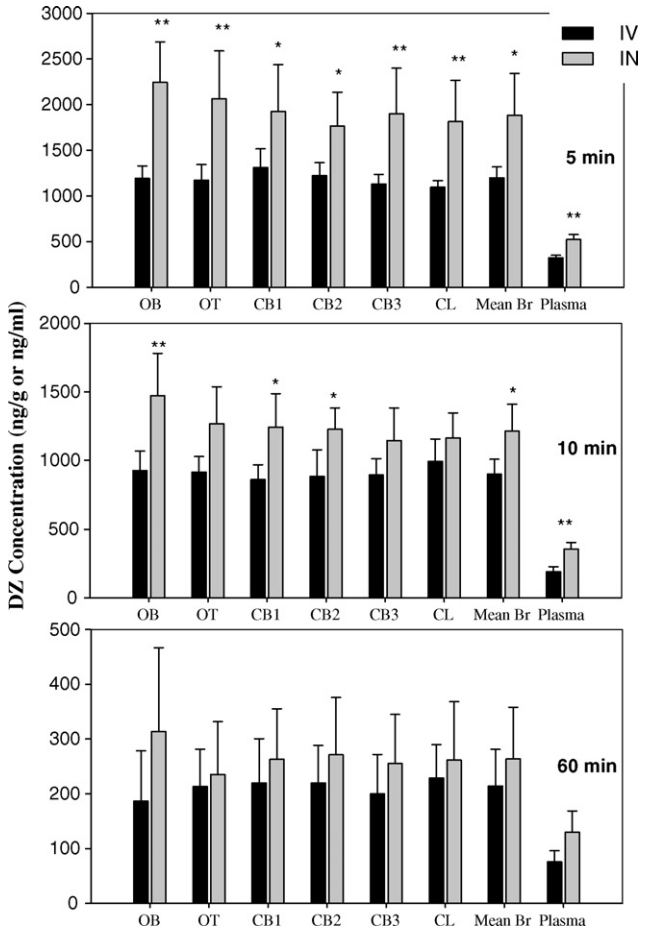

Fig. 9. DZ concentrations (ng/g or ng/mL) in various biological samples at 5, 10 and 60 min after IV (1 mg/kg) and IN (2 mg/kg) administration of DZ microemulsion in rats ($n = 5$ at each time point). $^{*}P < 0.05$, $^{**}P < 0.01$.

than rabbits may be due to the difference in the volume of the drug formulation applied to unit nasal epithelial area, since a greater volume of drug formulation (2.857 μL/cm^2) was administered per unit area of rat nasal mucosa as compared with that (1.639 μL/cm^2) applied to the rabbit nasal mucosa.

In order to evaluate whether there is a direct nose-to-brain transport pathway for DZ, the drug targeting efficiency defined as AUC_{brain}/AUC_{plasma} ratios calculated using the PK and distribution data generated after IV and IN administration in rabbits and rats are listed in Table 6. As shown in Table 6, in rats, the nearly identical AUC_{brain}/AUC_{plasma} ratios were obtained after IV (3.03 ± 0.07) and IN (3.00 ± 0.32) administration. However, in rabbits the AUC_{brain}/AUC_{plasma} values of DZ after IN (3.77 ± 0.17) administration were 12% lower than the AUC_{brain}/AUC_{plasma} values obtained after IV administration (4.23 ± 0.08). The homogeneous distribution patterns of the DZ seen into various regions of brain tissue after IN administration, and no higher drug targeting efficiency (DTE = AUC_{brain}/AUC_{plasma}) values obtained after IN administration

Table 5
Pharmacokinetic parameters of DZ after IV and IN administration of microemulsion formulation to rats

Route	Dose (mg/kg)	C_{max} (ng/mL g)	t_{max} (min)	$AUC_{0-60\,min}$ (ng h/mL g)	AUC_{IN}/AUC_{IV} (%)
Plasma–IV	1.0	556.8 ± 33.0	0	149.6 ± 18.1	100.0
Plasma–IN	2.0	522.2 ± 56.0	5	204.7 ± 26.2	68.4 ± 14.1
Brain–IV	1.0	1198.9 ± 118.8	5	454.4 ± 61.4	100.0
Brain–IN	2.0	1883.2 ± 456.4	5	615.6 ± 144.0	67.7 ± 12.2

Data are presented as mean ± S.D. ($n = 5$).

Table 6
AUC_{CSF}/AUC_{plasma} and AUC_{brain}/AUC_{plasma} of DZ after intravenous (IV) and intranasal (IN) administration of microemulsion (ME) formulation in rabbits ($n = 3$) and rats ($n = 5$)

AUC ratio	Rabbit		Rat	
	IV	IN	IV	IN
AUC_{CSF} (ng h/mL)	38.7 ± 6.3	42.1 ± 8.1	na	na
AUC_{plasma} (ng h/mL)	187.4 ± 13.8	193.3 ± 19.9	na	na
AUC_{CSF}/AUC_{plasma} [a]	0.21 ± 0.03	0.22 ± 0.02	na	na
AUC_{brain} (ng h/mL)	792.7 ± 44.2	728.4 ± 42.9	454.4 ± 61.4	615.6 ± 144.0
AUC_{plasma} (ng h/mL)	187.4 ± 13.8	193.3 ± 19.9	149.6 ± 18.1	204.7 ± 26.2
AUC_{brain}/AUC_{plasma} [a]	4.23 ± 0.08[a*]	3.77 ± 0.17[b*]	3.03 ± 0.07	3.00 ± 0.32

[a] $AUC_{brain\ or\ CSF}/AUC_{plasma}$ = drug targeting efficiency.
[*] $P < 0.05$.

compared with the IV administration indicate that the nasal administration of DZ microemulsion did not make any contribution to the direct brain delivery of the drug from nasal cavity in both animal models, i.e., rats and rabbits after IN administration. The results of this study are in agreement to the reported studies using low molecular weight and lipophilic compounds such as progesterone ($\log P = 4.03$) and estradiol ($\log P = 3.51$) (Van den Berg et al., 2004), a physostigmine analogue, NXX-066 ($\log P = 4.35$) (Dahlin and Björk, 2001), a serotonin antagonist, (S)-UH-301 ($\log P = 4.0$) (Dahlin and Björk, 2000), and dextromethorphan ($\log P = 3.87$) (Char et al., 1992), which have been shown to enter the CNS predominately by crossing the BBB after rapid absorption into the systemic circulation following nasal administration.

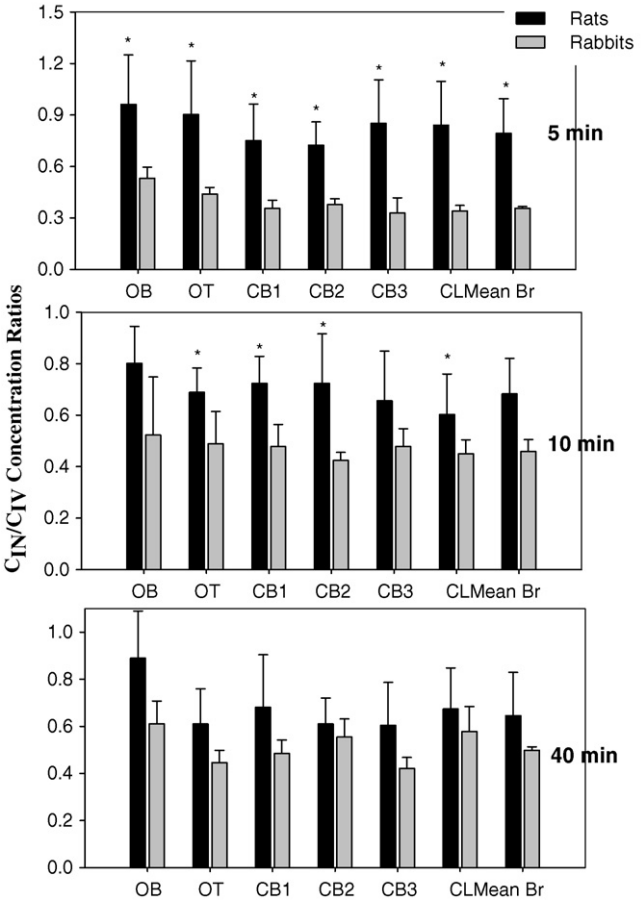

Fig. 10. C_{IN}/C_{IV} concentration ratios of DZ in different biological samples at 5, 10 and 40 min after IV (1 mg/kg) and IN administration of DZ microemulsion (2 mg/kg) to rats ($n = 5$) and rabbits ($n = 3$). The ratios are dose-normalized. [*] $P < 0.05$.

In order to examine the DTE differences in two animal models, the C_{IN}/C_{IV} concentration ratios calculated using the PK and distribution data determined in various regions of the brain tissue in rats and rabbits are shown in Fig. 10. Fig. 10 clearly showed that the C_{IN}/C_{IV} ratios obtained with various regions of brain tissue are significantly greater than those obtained with rabbits, in particular at the early time periods, i.e., 5 and 10 min. These interspecies differences at initial time points might be explained on the basis of difference in (i) experimental conditions, and (ii) olfactory epithelium area. For instance, DZ microemulsion was administered in a volume of 20 μL via a cannula inserted 10 mm into each nostril in anaesthetized rats. Although the nasal administration using the 10 mm cannula delivers the dose into the middle region of nasal cavity, but relatively low viscosity of the formulation (8.1 cps) and side position of the rats during dosing can promote the drug formulation reaching and covering the olfactory region. On the other hand, the nasal dosing of rabbits was performed in the conscious state held in rabbit restrainers at an angle of approximately 45° by spraying 50 μL of IN formulation into each nostril with a Pfeiffer spray device. The formulations delivered as spray deposit in the anterior region of the nasal cavity. Therefore, the observed differences might be partly explained on the basis of difference in the deposition pattern, location and the position of animal during administration. In rats, the olfactory epithelium occupies a large area (≅50%) of the total nasal epithelium, whereas in rabbits olfactory epithelium covers a small area (≅10%) of nasal cavity. Furthermore, in rats, the olfactory area is spread throughout the nasal cavity. Therefore, an increased contact of the DZ molecules with the olfactory mucosa can also account for observed difference in DTE values and C_{IN}/C_{IV} ratios at initial time points, i.e., 5 and 10 min. However, the current results in rabbits and rats suggest that contribution of direct nose-to-brain transport of DZ molecules was not significantly affected by the difference in percentage of olfactory epithelium covering nasal mucosa.

4. Conclusions

In vivo absorption and brain distribution studies in rabbits revealed that DZ was rapidly absorbed into the systemic circulation and readily distributed into the brain tissues after IN administration of DZ microemulsion with a t_{max} of 5–10 min. The brain distribution studies in rabbits and rats suggest that the drug molecules were mainly transported through the blood–brain barrier after a fast absorption of drug into the blood after IN administration. No significant direct nose-to-brain transport was detected for a lipophilic compound DZ in rats and rabbits after IN dosing in spite of difference in the percentage of olfactory epithelium covering nasal mucosa. In addition, the ethyl laurate-caprylcaproyl macrogol-8 glyceride based DZ microemulsion system showing a t_{max} of 2–10 min and bioavailability of 51–68% is a very promising approach to the development of a rapid onset intranasal delivery system of DZ for use in the treatment of convulsive attack phenomena of epilepsies.

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References

Bechgaard, E., Gizurarson, S., Hjortkjaer, R.K., 1997. Pharmacokinetic and pharmacodynamic response after intranasal administration of diazepam to rabbits. J. Pharm. Pharmacol. 49, 747–750.

Char, H., Kumar, S., Pater, S., Piemontese, D., Iqbal, K., Malick, A.W., Salvodor, R.A., Behl, C.R., 1992. Nasal delivery of [^{14}C] dextromethorphan hydrochloride in rats: levels in plasma and brain. J. Pharm. Sci. 81, 750–752.

Chow, H.H.S., Chen, Z., Matsuura, G.T., 1999. Direct transport of cocaine from the nasal cavity to the brain following intranasal cocaine administration in rats. J. Pharm. Sci. 88, 754–758.

Dahlin, M., Björk, E., 2000. Nasal absorption of (S)-UH-301 and its transport into cerebrospinal fluid of rats. Int. J. Pharm. 195, 197–205.

Dahlin, M., Björk, E., 2001. Nasal administration of a physostigmine analogue (NXX-066) for Alzheimer's disease to rats. Int. J. Pharm. 212, 267–274.

Einer-Jensen, N., Larsen, L., 2000. Local Transfer of Diazepam, but Not of cocaine, from the nasal cavities to the brain arterial blood in rats. Pharmacol. Toxicol. 87, 276–278.

Gizurarson, S., 1990. Animal models for intranasal drug delivery studies. Acta Pharm. Nordica. 2, 105–122.

Illum, L., 2000. Transport of drugs from the nasal cavity to the central nervous system. Eur. J. Pharm. Sci. 11, 1–18.

Kilpatrick, C., 1999. Benzodiazepines. In: Eadie, J.M. (Ed.), Antiepileptic Drugs: Pharmacology and Therapeutics. Springer-Verlag, New York, pp. 399–406.

Li, L., Gorukanti, S., Choi, M.Y., Kim, K.H., 2000. Rapid-onset intranasal delivery of anticonvulsants: pharmacokinetic and pharmacodynamic evaluation in rabbits. Int. J. Pharm. 199, 65–76.

Li, L., Nandi, I., Kim, K.H., 2002. Development of an ethyl laurate-based microemulsion for rapid-onset intranasal delivery of diazepam. Int. J. Pharm. 237, 77–85.

Lindhardt, K., Olafsson, R.D., Stefansson, B.S., Bechgaard, E., 2002. Intranasal bioavailability of diazepam in sheep correlated to rabbit and man. Int. J. Pharm. 231, 62–72.

Lott, R.S., 1990. Seizure disorders. In: Koda-Kimble, A.M. (Ed.), Applied Therapeutics: The Clinical Use of Drugs. Vancouver, Washington, pp. 1369–1396.

McNamara, J.O., 1996. Drugs effective in the therapy of the epilepsies. In: Gilman, A.G. (Ed.), The Pharmacological Basis of Therapeutics. McGraw Hill, New York, pp. 461–486.

Meisami, E., Louie, J., Hudson, R., Distel, H., 1990. A morphometric comparison of the olfactory epithelium of newborn and weanling rabbits. Cell. Tissue Res. 262, 89–97.

Treiman, M.D., Walker, C.M., 2006. Treatment of seizure emergencies: convulsive and non-convulsive status—epilepticus. Epilepsy Res. 688, 577–582.

Tedeschi, G., Smith, A.T., Dhillon, S., Richens, A., 1983. Rate of entrance of benzodiazepines into the brain determined by eye movement recording. Br. J. Clin. Pharmacol. 15, 103–107.

Van den Berg, P.M., Verhoef, C.J., Romeijn, G.S., Merkus, W.H.M.F., 2004. Uptake of estradiol or progesterone into the CSF following intranasal and intravenous delivery in rats. Eur. J. Pharm. Biopharm. 58, 131–135.