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Rapid-onset intranasal delivery of anticonvulsants: pharmacokinetic and pharmacodynamic evaluation in rabbits

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Abstract

Intranasal (IN) administration is a promising approach for rapid-onset delivery of medications. In order to assess the feasibility of this approach for the emergency treatment of status epilepticus, three anticovulsants, i.e. diazepam (DZ), clonazepam (CZ), and a monocarbamate-based new compound (MCA) were studied in rabbits for the pharmacokinetics (PK) and pharmacodynamic (PD) response following intravenous (IV) and IN administrations. The animals were intranasally dosed with DZ (1 mg/kg), CZ (0.2 mg/kg), and MCA (5 mg/kg), dissolved in 200 μl of vehicle consisting of propylene glycol (PG), ethanol (EtOH), and water in the presence or absence of 1% sodium glycocholate (SGC) using single and repeated dosing schedules. Both DZ and CZ were absorbed very rapidly from 1% SGC/60% PG-30% EtOH-10% Water after IN single application; the T_{max}'s were less than 2 min. The absorption rate of MCA was relatively slower with the peak time of 13–32 min. The bioavailability of single IN administration for DZ, CZ, and MCA determined over the first 2 or 4 h was found to be 77, 45, and 79%, respectively. The peak plasma level of DZ increased linearly with increasing the volume fraction of EtOH in the ternary cosolvent (20% to 60%). A repeated IN application of DZ, 5 min after the first dose, doubled the C_{max} and $AUC_{0-2\,h}$ values of the first one, whereas those of CZ and MCA resulted in an increase of 73–94% of the first dose. A single IN application of DZ- and CZ-containing formulations produced a PD response within 1.5 min, which was comparable to that of an IV injection. These results suggest that single or repeated IN applications of DZ, CZ, and MCA in a hydroalcohol-glycolic formulation might represent a viable approach to achieving a rapid systemic absorption of these anticonvulsants during the emergency treatment of status epilepticus and other types of seizures.

Keywords: Diazepam; Clonazepam; Monocarbamate derivative; Intranasal administration; Repeated intranasal dosing; Status epilepticus

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1. Introduction

Status epilepticus is a serious and life-threatening neurological emergency, which requires prompt treatment to minimize mortality as well as to reduce the risk of permanent brain damage caused by prolonged seizure activity (Meldrum, 1978).

Currently several drugs are available to treat this neurological disorder, of which DZ and lorazepam are the most widely used two benzodiazepines for this purpose (Homan and Walker, 1983; Leppik et al., 1983). The intravenous administration of anticonvulsants is probably the most rapid way to suppress epileptic convulsions. However, other routes of administration would appear more desirable when IV administration is inconvenient and delaying, for instance, because of technical difficulties such as requirement for sterile equipment and skilled personnel. In addition, IV dosing is often associated with hypotension, cardiac dysrhythmia, and respiratory failure due to an excessive blood concentration (Lott, 1990). In this respect, a recent human PK study of DZ after intramuscular, oral and rectal administration showed that only the later route provided a fairly rapid absorption and thus, it might be looked upon as an alternative route to IV injection (Moolenaar et al., 1980). However, the rectal route is a very inconvenient way of drug administration particularly in emergency situation. More recently, the nasal route has received a great deal of attention as a convenient and reliable mode for the systemic administration of drugs. In addition, the epithelial cells of nasal mucosa are highly vascularized, and thus it provides an attractive site for rapid and efficient systemic absorption of drugs including peptides and proteins. Nevertheless, one of the biggest limitations of the intranasal drug delivery is insufficient drug absorption through the nasal mucosa. Many promising drug candidates can not be developed for the nasal route because they are not absorbed well enough to produce therapeutic effects. An additional constraint concerning nasal administration is that a small administration volume is required; it is not generally possible to administer more than approximately 150 μl per nostril; above this volume the formulation will be drained out into the pharynx and swallowed (Gizurarson, 1993). Therefore, a great need exists for solvent vehicles, in which the solubility of the drug is high and which, on the other hand, are nonirritating to the nasal mucosa. The solubility problem is even more critical for a very poorly water-soluble drug such as DZ and CZ. Adding a chemical adjuvant or permeation enhancer, including bile salts, can increase the intranasal absorption of drugs. For example, Lau and Slattery (1989) attempted to administer a benzodiazepine such as DZ or lorazepam by dissolving the drugs in solvents such as triacetin, dimethylsulfoxide, polyethylene glycol 400, polyoxyethylated castor oil (Cremophor EL), laureth-9 polyoxyethylene-9 lauryl ether (Lipal-9-LA), isopropyl adipate and Azone. Although many of the solvents dissolved DZ and lorazepam in the desired concentrations, they were too irritating to be used, when administered to the nose. Cremophor EL was found to be the least irritant for nasal mucosal tissue, but with this vehicle the nasal absorption of DZ in humans was rather slow ($T_{max} = 1.4$ h) and plasma peak concentration was much lower than that observed after IV administration. The IV administration of benzodiazepines such as midazolam and flurazepam in dogs showed that the T_{max} was attained within 15 min (Lui et al., 1991) and similar PK results were obtained with midazolam in children (Malinovisky et al., 1993). Studies on rectal administration of DZ showed that the therapeutic concentration of the drug was achieved at approximately 20 min, which was sufficiently rapid to control seizures in most patients suffering from multiple seizures (Knudsen, 1979; Kriel et al., 1991). Most recently, Bechgaard et al. (1997) reported the use of pure organic solvents such as glycofurol and tetraethyleneglycol, and their combinations as carriers for the nasal delivery of DZ. The absolute bioavailability, measured during the first 30 min after the nasal administration, was 49–62% for the most promising carrier systems, and the rapid pharmacodynamic response was found within 3.5 min for these systems. Based on the clinical and PK results, an effective treatment within 5 min is considered to be an attractive goal in the prompt management of status epilepticus.

The present study was carried out to investigate the absorption characteristics and PD response of three anticonvulsants, the benzodiazepines, DZ and CZ, and the new monocarbamate anticonvulsant, MCA after their IN administration as solutions in aqueous cosolvent system containing various proportions of PG and EtOH in the presence of 1% SGC. The aqueous ternary cosolvent vehicles, prepared with GRAS materials, provided a sufficient solubility and chemical stability for the anticonvulsants in the preformulation studies. These vehicles were found to possess an acceptable flavor and palatability. In addition, the preliminary local toxicity evaluation with SEM showed that the IN vehicles exhibited a minor local irritation when administered to the nasal mucosa of rabbits. A repeated IN dosing regimen was also investigated in the present study with a view to attaining therapeutic concentrations of the drugs within a short time interval if the single application is incapable of producing the desired antiseizure effect.

2. Materials and methods

2.1. Chemicals

DZ and CZ were purchased from Sigma (St. Louis, MO). MCA and MCA-1 were kindly supplied from SK Corporation (Fairfield, NJ). DZ injection was obtained from Elkins-Sinn, Inc. (Cherry Hill, NJ). Polyethylene glycol 400 (PEG 400), PG, SGC, diethyl ether, phosphoric acid, and ethylenediaminetetracetic acid (EDTA) were purchased from Sigma (St. Louis, MO). EtOH was obtained from Quantum Chemical Co. (Newark, NJ). Acetonitrile and monobasic potassium phosphate were purchased from EM Science (Gibbstown, NJ). Methanol, methyl-*t*-butyl ether, perchloric acid, and sodium hydroxide were obtained from J.T. Baker Chemical Co (South Plainfield, NJ). Polyoxyethylated castor oil (Cremorphor EL) was obtained from PVO Research (Boonton, NJ). The water was deionized and distilled in glass.

2.2. Animals

New Zealand white rabbits (2.5–4.0 kg), obtained from Marland Breeding Farms, Inc. (Hewitt, NJ), were used in the PK and PD studies with a washout period of 14 days. Animals were housed in individual cages with free access to food and water. In vivo experiments were performed after an acclimation period of at least 2 weeks to the new environment, a room with an automatically controlled illumination (a 12-h light–dark cycle), temperature, and relative humidity.

2.3. Test formulations

DZ for IV injection (5 mg/ml) was used as received. CZ (1.5 mg/ml) and MCA (20 mg/ml) solutions were prepared under aseptic conditions for IV injections using a 40% PG–30% EtOH–30% water cosolvent or 40% PEG 400 aqueous solution, respectively. For IN application, the test formulations were prepared in 30% EtOH–60% PG–10% water, plus or minus 1% SGC. All the nasal formulations were prepared just prior to the experiment by dissolving DZ (20 mg), CZ (4.2 mg), or MCA (100 mg) in 1 ml of the aforementioned vehicles. To evaluate the effect of vehicle composition on the PK characteristics of DZ, drug solutions (20 mg/ml) were prepared using an SGC-containing ternary cosolvent systems that included 30–70% PG, 20–60% EtOH, and 10% water. Another nasal formulation containing DZ (20 mg/ml) and a nonionic surfactant of polyoxyethylenated castor oil (Cremophor EL), was also prepared for comparative purposes according to Lau and Slattery (1989).

2.4. PK experimental procedures

Just prior to the experiment, rabbits ($n = 2$–4) were weighed and restrained in rabbit restrainers. In the IV treatment, the rabbits received DZ (1 mg/kg), CZ (0.2 mg/kg), and MCA (5 mg/kg) through a marginal ear vein over 20 s. For IN administration, each rabbit received 100 μl of the IN formulations into each nostril within 5 s by means of a Pfeiffer spray device. For the repeated dosing studies, the same volume of the IN formu-

lation (100 μl) was sprayed into each nostril 5 min after the first dose. The Cremophor EL-containing formulation was delivered using a Pfeiffer gel spray device. Blood samples (0.5–1 ml) were collected just prior to dosing and at 2, 5, 10, 20, 30, 45, 60, and 120 min after IV and IN administration of DZ and CZ. For MCA studies, the blood sample collections were extended 180 and 240 min after dosing. All the blood samples were delivered into EDTA-treated test tubes, and plasma samples were separated by centrifugation and stored at −20°C until analysis.

2.5. Analytical procedures

Plasma DZ concentrations were analyzed by HPLC after precipitating the plasma samples with 0.01% (v/v) perchloric acid solution in acetonitrile. The above precipitating solution (250 μl) containing CZ as the internal standard at a concentration of 1 μg/ml was added to the plasma sample (250 μl). The mixture was vortex-mixed for 30 s and centrifuged at 4000 rpm for 10 min. An aliquot of the supernatant solution (100 μl) was injected into an HPLC system consisting of a 600E multisolvent delivery system, a 717 Plus autoinjector, a 996 photodiode array detector, and a 2010 Millenium data management system (Waters Corporation, Milford, MA). A reversed phase Symmetry C_{18} column (15 cm × 3.9 mm I.D. × 5 μm; Waters Corporation) was used at room temperature. The mobile phase was 50% methanol/10% acetonitrile/40% pH 3.5 0.03 M KH_2PO_4/H_3PO_4 buffer by volume. The flow rate of the mobile phase was 1 ml/min and detection was at 229 nm. Under these analytical conditions, the detection limit for DZ was found to be 70 nmol/l.

For the determination of plasma CZ concentrations, 0.5 M NaOH (50 μl) and internal standard working solution (5 μg/ml DZ in methanol; 10 μl) were added to the plasma (500 μl) and thoroughly mixed. To the above mixture, diethyl ether (5 ml) was added as an extracting solvent. The sample was vortex-mixed for 1 min and centrifuged at 4000 rpm for 10 min. A portion of the ether layer was transferred to a test tube and evaporated to dryness at 40°C by means of a vacuum evaporator. The residue was dissolved in 100 μl of the mobile phase, and 50 μl of the solution was injected into the HPLC system. The mobile phase was prepared with 20% methanol–30% acetonitrile-50% pH 3.5 0.03 M KH_2PO_4/H_3PO_4 buffer solution. The flow rate was 1 ml/min, and detection was at 254 nm. Under these analytical conditions, the detection limit for CZ was 16 nmol/l.

For the analysis of plasma MCA concentrations, internal standard working solution (10 μg/ml MCA-1 in deionized water; 50 μl) was added into the plasma sample (500 μl), and then methyl butyl ether (5 ml) was added as an extracting solvent. The mixture was vortex-mixed thoroughly for 1 min, and then centrifuged at 4000 rpm for 10 min. A portion of the ether layer was transferred to a test tube and evaporated to dryness at 40°C under reduced pressure. The residue was dissolved in 200 μl of deionized water, and 150 μl of the solution was injected into the HPLC system. The mobile phase used in this case was prepared with 20% acetonitrile–80% water. The flow rate was 1 ml/min, and detection was made at 210 nm. Under these analytical conditions, the detection limit for MCA was 23 nmol/l.

2.6. PD response tests

The PD response was examined in rabbits by evaluating the muscle relaxation effects of DZ and CZ after IV and IN administration following the method reported by Bechgaard et al. (1997). The parenteral solutions of DZ and CZ, which were used in the PK studies, were examined in the PD study. The IN formulations prepared with 1% SGC/60% PG–30% EtOH–10% water as the vehicle were used in this study. For comparison, the Cremophor EL-based formulation of DZ was also examined in the PD study. The dosing levels were also the same as used in the PK studies, i.e. 1 mg/kg for DZ and 0.2 mg/kg for CZ. The pharmacological response was measured after the application of 100 μl of nasal formulations into each nostril of rabbits using a Pfeiffer spray pump. Immediately after dosing the rabbit was laid down with both hind legs to one side, and then firmly tipped by a finger on the hip. The mean time for the rabbits to start remaining in the laid down

position without trying to stand up was measured as the PD response time.

2.7. PK calculation and statistics

All plasma concentration data were dose- and weight-corrected. The PK parameters were analyzed using WinNonlin noncompartmental program (Scientific Consulting Inc., Apex, NC). The area-under-the curve (AUC_{0-t}) was determined by the linear trapezoidal method in the program. The C_{max} values following IV injections were estimated by extrapolating the initial plasma drug concentration–time curve to the Y-axis at time zero in the program (C_0). The bioavailability (F%) of drugs after single or repeated IN administration was calculated using the following equations:

$$F_{single}\ (\%) = [(AUC_{0-t,\ IN,\ single})/(AUC_{0-t,\ IV,\ single})] \times 100;$$

$$F_{double}\ (\%) = [(AUC_{0-t,\ IN,\ double})/(2 \times AUC_{0-t,\ IV,\ single})] \times 100;$$

$$F_{triple}\ (\%) = [(AUC_{0-t,\ IN,\ triple})/(3 \times AUC_{0-t,\ IV,\ single})] \times 100.$$

The indirect parameter, $C_{max,\ IN}/AUC_{0-t,\ IN}$ (Lacey et al., 1994) was utilized to evaluate the rate of absorption after IN administration. Statistical analysis was performed utilizing standard method; Student's t–test was employed for calculating the significance.

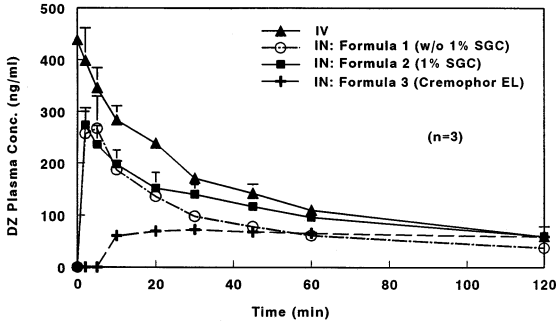

Fig. 1. Mean plasma concentration-time profiles of DZ after single IV and IN administration of the drug solutions at 1 mg/kg dose in rabbits

3. Results and discussion

3.1. Intranasal absorption of DZ

The time courses of the mean plasma levels of DZ obtained after single and double IN administration of the drug in various formulations and single IV injection are presented in Fig. 1. The corresponding bioavailability data and PK parameters derived from these plasma levels are summarized in Table 1. As shown in Table 1 and Fig. 1, after single dosing, IN formula 2, which was prepared using a ternary cosolvent consisting of 30% EtOH–60% PG–10% water in the presence of 1% SGC, increased significantly the transnasal absorption of DZ ($P < 0.05$) when compared with the Cremophor EL-based formula 3. The IN formula 2 produced the peak plasma concentration within 2 min and the C_{max} was calculated to be 62% of that of IV injection at the corresponding time (2 min). The bioavailability obtained with the IN formula 2 was approximately 77% ($AUC_{0-2\ h,\ IN}$, 226.7 ng × h/ml vs. $AUC_{0-2\ h,\ IV}$, 293.1 ng × h/ml). On the other hand, in the case of IN administration of the Cremophor EL-based formula 3, the peak plasma concentration occurred at 35 min, which was about 7.5 times slower than that of the formula 2. The C_{max} and bioavailability values for the Cremophor EL formulation were about 17 and 42% of those obtained by IV administration, respectively. The comparative bioavailability data obtained with two different IN formulations indicate that the hydroalcoholic ternary cosolvent was highly effective in enhancing the nasal absorption of a hydrophobic compound, DZ when compared with a lipophilic surfactant Cremophor EL carrier. In addition, the slow rate of absorption and low bioavailability data observed in the present study supported the human PK data reported by Lau and Slattery (1989). The Cremophor EL formulation yielded a slow rate of absorption in humans (T_{max} of 1.4 h) after IN administration

Table 1
Bioavailabilities and pharmacokinetic parameters after IV and IN administration of a single or double 1 mg/kg dose of DZ in various formulations to rabbits

Route/formulation	Dosing	C_{max} (ng/ml)[g]	T_{max} (min)	$C_{max}/AUC_{(0-2\ h)}$ (1/h)	$AUC_{(0-2\ h)}$ (ng × h/ml)	F (%)
IV formula[a]	Single	438.6 (84.7)	0.0	1.50 (0.31)	293.1 (7.0)	100.0
IN formula 1[b]	Single	268.7 (61.1)	4.0 (1.7)	1.55 (0.35)	173.1 (11.5)	59.1 (3.9)
IN formula 2[c]	Single	273.6 (26.4)	2.0 (0.0)	1.21 (0.13)	226.7 (12.2)	77.3 (4.2)
IN formula 2[c]	Double[e]	625.7 (14.7)	13.3 (5.8)	1.43 (0.28)	446.5 (81.0)	76.2[f] (13.8)
IN formula 3[d]	Single	73.5 (11.9)	35.0 (8.76)	0.61 (0.01)	122.6 (20.3)	41.8 (6.93)

[a] IV formula, 0.5% diazepam injection, USP, Elkins-Sinn, Inc.
[b] IN formula 1, 2% diazepam solution in 60% PG–30% EtOH–10% water.
[c] IN formula 2, 2% diazepam solution in 1% SGC/60% PG–30% EtOH–10% water.
[d] IN formula 3, 2% diazepam solution in Cremophor EL.
[e] Interval between doses, 5 min.
[f] Normalized data using the following equation, $F = [(AUC_{IN,\ 1\ mg/kg,\ double})/(2 \times AUC_{IV,\ 1\ mg/kg,\ single})] \times 100$.
[g] Values given in parenthesis are the standard deviations for $n = 3$.

and the C_{max} was only 27% relative to the IV injection.

Bile salts are the most widely used surfactants for the enhancement of nasal absorption of drugs. At relatively low concentrations of 10–20 mmol, they are able to improve the absorption of drugs (Behl et al., 1998). Among the commonly used biological surfactants, SGC has been one of the most extensively employed bile salts in in vitro and in vivo studies. In the present study, SGC was utilized in a concentration (1%w/v) known to enhance intranasal absorption while producing minimal irritation and morphological alteration in the nasal mucosa (Su et al., 1985; Gizurarson et al., 1990). The results presented in Table 1 show that the addition of 1% SGC to the hydroalcoholic cosolvent produced an approximately 31% increase in the IN bioavailability of DZ as compared with that of the IN formulation without the bile salt ($P < 0.05$). The precise mechanisms of enhancer effects have not been well defined. However, it is generally believed that some enhancers such as surfactants, bile salts, microparticulates and cosolvents show their actions by altering the drug solubility, drug partition coefficient, and/or weak ionic interactions with the drugs (Hirai et al., 1985). This mechanism of drug absorption enhancement is desirable because it can be effective with the lowest potential of toxicity (Behl et al., 1998) Another possible mechanisms by which bile salts and cosolvents produce their promoting effects include formation of aqueous pore-type transport pathways (Gordon et al., 1985) and protein and phospholipid leaching from the epithelial cells of nasal mucosa, which may cause local irritation to the mucous layer (Hirata et al., 1979; Hosoya et al., 1994).

As described earlier, the IN formulas exhibited a minor irritation when administered in the nasal cavity in the preliminary toxicological evaluation using SEM. Further well-designed animal toxicological studies should be performed to characterize the mechanism underlying the formulation's effect on the mucous membrane. The final decision should take into consideration the therapeutic benefit to risk ratio, since the IN formulation is intended primarily for the emergency use in the treatment of status epilepticus.

In an effort to characterize the absorption properties of DZ in the multiple IN administration within a short time interval, a repeated dosing regimen was investigated using the IN formula 2 at a 5 min interval. The PK profiles determined after single and double IN administrations in rabbits are presented in Fig. 2 and the PK parameters derived from the plasma levels are tabulated in Table 1. The results clearly show that the C_{max} and $AUC_{0-2\ h}$ values attained after the second application of the same dose doubled those generated with the first one. These data

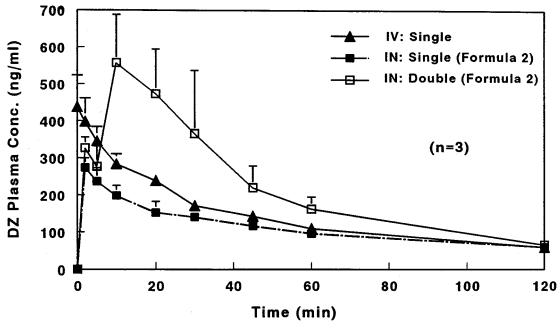

Fig. 2. Mean plasma concentration-time profiles of DZ after single IV and single and double IN administration of the drug solutions at 1 mg/kg dose in rabbits.

clearly indicate that the second intranasal dose produced an essentially identical rate and extent of absorption of the drug to those obtained from the first application. In addition, the plasma DZ level reached after the second IN dosing exceeded that of single IV administration within 7 min. These findings strongly suggest that a repeated IN dosing regimen (within a short time interval) might be effectively utilized for the acute management of medical emergency such as epileptic seizures when a single dosing was incapable of producing the desired therapeutic effect. In addition, the single and repeated IN delivery of DZ might be of clinical importance as an auxiliary sedation in surgical procedures. For the determination of optimal time interval for the repeated IN administration, additional PK studies are required since the absorption efficiency of drugs from the nasal mucosa is significantly affected by the contact time between the drug molecules and epithelial tissues. Normal human mucociliary transit time has been reported to be 12–15 min (Schipper et al., 1991). Several other studies in animals and humans have shown that drugs including peptides and proteins are predominantly absorbed by the nasal epithelium within 5–15 min (Marttin et al., 1998). Based on the reported residence time of drugs in the nasal cavity and the PK data obtained with DZ after the second dosing, the 5 min time interval employed in the present study was considered to be fairly appropriate for producing an effective absorption of the drug through the rabbit nasal mucosa.

3.2. Effect of EtOH/PG volume fractions in the IN formulation

The effect of the volume fractions of both EtOH and PG on the pharmacokinetics of DZ after IN administration was studied using the ternary cosolvent formulation containing 1% SGC. In this case, the volume percentages of EtOH, PG and water incorporated into the test preparations were varied to represent 20/70/10, 30/60/10, and 60/30/10, respectively. The PK parameters determined after single IN administration of these formulations to rabbits are summarized in Table 2. The peak DZ plasma concentration, observed within 2–3 min after IN

Table 2
Effect of EtOH/PG volume ratio of the IN formulations on the pharmacokinetic parameters of DZ after IV and IN administration of a single 1 mg/kg dose to rabbits

Route/formulation	Dosing	C_{max} (ng/ml)	T_{max} (min)	$C_{max}/AUC_{(0-2\ h)}$ (1/h)	$AUC_{(0-2\ h)}$ (ng × h/ml)	F (%)
IV formula[a]	Single	438.6 (84.7)	0.0	1.50 (0.31)	293.1 (7.0)	100.0
IN formula A[b]	Single	313.1 (17.3)	2.0 (0.0)	1.38 (0.04)	226.8 (6.5)	77.4 (2.2)
IV formula B[c]	Single	273.6 (26.4)	2.0 (0.0)	1.21 (0.13)	226.7 (12.2)	77.3 (4.2)
IV formula C[d]	Single	250.7 (26.2)	3.0 (1.7)	1.18 (0.20)	214.3 (13.8)	73.1 (4.7)

[a] IV formula: 0.5% diazepam injection, USP, Elkins-Sinn, Inc.
[b] IN formula A: 2% diazepam solution in 1% SGC/30% PG–60% EtOH–10% water.
[c] IN formula B: 2% diazepam solution in 1% SGC/60% PG–30% EtOH–10% water.
[d] IN formula C, 2% diazepam solution in 1% SGC/70% PG–20% EtOH–10% water. Values given in parenthesis are the standard deviations for $n = 3$.

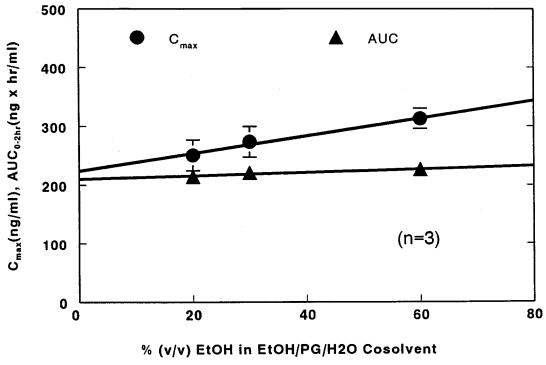

Fig. 3. Effect of ETOH concentration in the 1% SGC incorporated EtOH–PG–water cosolvent vehicle on the C_{max} and $AUC_{0-2\ h}$ of DZ after single IN administration of the drug solutions.

administration, increased significantly with increasing EtOH concentrations in the IN formulations (20–60%) ($P < 0.05$), but the $AUC_{0-2\ h}$ showed only a slight upward trend. A linear relationship between the C_{max} and the percentage volume of ETOH present in the IN preparations was shown in Fig. 3. When considered together, these results indicate that the peak plasma level of the drug might be controllable through changes in the EtOH/PG volume ratio of the IN formulation.

3.3. Intranasal absorption of CZ

The mean plasma concentration-time profiles of CZ following single IV and single, double, and triple IN administration of the drug solution in the ternary cosolvent-based formulation containing 1% SGC are presented in Fig. 4. The corresponding bioavailability data and other relevant PK parameters determined from the plasma levels of CZ are listed in Table 3. A single nasal application of CZ yielded peak plasma level within 2 min, which was only about 30% of that by the IV route, and an absolute bioavailability of 45%. This bioavailability appeared to be significantly lower than that achieved with the DZ formulation (Table 1), probably due to the difference in the lipophilic nature of the two compounds. In this context, a more lipophilic benzodiazepine, DZ ($K_{oct/water} = 702.3$) produced a greater extent of absorption through the nasal mucosa than CZ ($K_{oct/water} = 199.3$). In the case of repeated applications, the C_{max} and $AUC_{0-2\ h}$ of CZ increased in a linear fashion with the number of dose (0.2 mg/kg) administered (i.e. one, two and three times with a 5 min interval). The times to reach the peak levels after the single, double, and triple applications were 2.0, 4.7, and 15.0 min, respectively. The plasma level attained after the triple application was found to be nearly identical to that of single IV administration of CZ. In addition, the relative bioavailabilities of CZ after the second and third dosing was 73 and 77% of that of the first dosing, respectively, suggesting that the absorption efficiency of the drug solution somewhat decreased by the repeated IN application. Another noteworthy finding from the comparative PK profiles of CZ after the IV and IN administration of CZ solutions was the significant difference in the elimination phases of CZ between the two different modes of administration. The plasma concentration of CZ after IV administration declined rapidly, with a mean half-life of approximately 2.5 h, whereas the single and multiple IN administration exhibited a slow and sustained elimination pattern with the mean half-life of approximately 11.6 h. The slow elimination rate profiles observed after the IN application suggest that CZ might be absorbed through the nasal mucosa over a long period of time and/or the drug molecules might be long retained by the nasal epithelial tissues during the absorption process and diffused slowly into the blood stream.

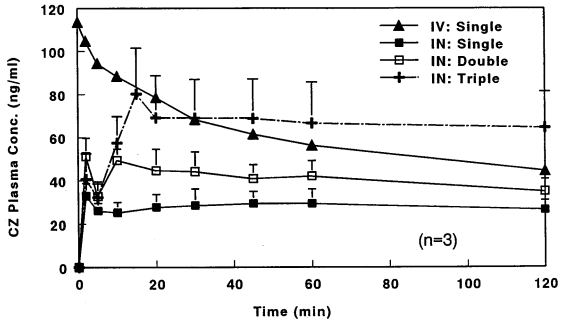

Fig. 4. Mean plasma concentration-time profiles of CZ after single IV and single, double, and triple IN administration of the drug solutions at 0.2 mg/kg dose in rabbits.

3.4. Intranasal absorption of MCA

MCA is a new aromatic monocarbamate antiepileptic compound, which is currently under development. In the preliminary pharmacological screening, this compound demonstrated a potent anticonvulsant activity against both electrically- and chemically-induced seizures in rodents. The bioavailability and pharmacokinetic data determined after single IV and IN administration of MCA solutions in EtOH/PG/water based ternary cosolvent containing 1% SGC at the 2.5 and 5 mg/kg doses in rabbits are summarized in Table 4. After a single IN administration, the peak concentrations for MCA, attained within 12.5 and 31.7 min, were found to increase proportionally to the dose administered. Based on the AUC data determined over 0–4 h time period, the bioavailability of MCA nasal solution in the ternary cosolvent vehicle was found to be 72–79% for the two doses examined. Under the same conditions, the C_{max} values were in the range of 30–37% of those of IV injections. The PK profiles generated after single and double IN applications of a 5 mg/kg dose of MCA are illustrated in Fig. 5. The relevant PK parameters determined from the blood levels are summarized in Table 4. The PK data demonstrate that a second IN application of an MCA formulation 5 min after the first one, produced a high absorption efficiency, which was comparable to that observed earlier with the DZ intranasal formulation Fig. 6. The relative bioavailability and peak plasma level achieved after the second dosing were 94 and 89% of the first dosing, respectively. In addition, the plasma concentration of MCA achieved after the second IN administration exceeded the plasma drug level of the single IV injection within 30 min. These results also support the effective utilization of the repeated IN application to produce a fairly rapid therapeutic effect by simple and convenient nasal spray in the treatment of medical emergency.

3.5. PD response after IN application of DZ and CZ

Benzodiazepines such as diazepam and clonazepam are known to possess the following pharmacological effects in animal models: (i) relief of anxiety, (ii) central depressant action on spinal reflexes, (iii) anticonvulsive action, and (iv) muscle relaxation (Baldessarini, 1996). Considering the relevance of the PD evaluation, anticonvulsant tests such as maximal electroshock seizure (MES) and hippocampal kindling tests would be more appropriate for the evaluation of the IN formulations. In the present study, however, the muscle relaxation test method

Table 3
Bioavailabilities and pharmacokinetic parameters after IV and IN administration of a single, double, or triple 0.2 mg/kg dose of CZ to rabbits

Route/formulation	Dosing	C_{max} (ng/ml)[f]	T_{max} (min)	$C_{max}/AUC_{(0-2\ h)}$ (1/h)	$AUC_{(0-2\ h)}$ (ng × h/ml)	F (%)
IV formula[a](n = 2)	Single	112.3	0.0	0.91	123.9	100.0
IN formula[b](n = 3)	Single	33.0 (5.9)	2.0 (0.0)	0.59 (0.05)	55.9 (11.1)	45.1 (9.0)
IN formula[b](n = 3)	Double[c]	52.1 (7.3)	4.7 (4.6)	0.64 (0.02)	81.3 (13.9)	32.8[d] (5.6)
IN formula[b](n = 3)	Triple[c]	80.2 (21.3)	15.0 (0.0)	0.62 (0.04)	129.4 (34.6)	34.8[e] (9.3)

[a] IV formula: 0.15% clonazepam solution in 40% PG–30% EtOH–30% water.
[b] IN formula: 0.42% clonazepam solution in 1% SGC/60% PG–30% EtOH–10% water.
[c] Interval between doses: 5 min.
[d] Normalized data using the following equation: $F = [(AUC_{IN,\ 0.2\ mg/kg,\ double})/(2 \times AUC_{IV,\ 0.2\ mg/kg,\ single})] \times 100$.
[e] Normalized data using the following equation: $F = [(AUC_{IN,\ 0.2\ mg/kg,\ triple})/(2 \times AUC_{IV,\ 0.2\ mg/kg,\ single})] \times 100$.
[f] Values given in parenthesis are the standard deviations for $n = 3$.

Table 4
Bioavailabilities and pharmacokinetic parameters after IV and IN administration of a single or double dose (2.5 or 5 mg/kg) of MCA to rabbits

Route/formulation	Dosing (mg/kg)[f]	C_{max} (ng/ml)	T_{max} (min)	$C_{max}/AUC_{(0-4\ h)}$ (1/h)	$AUC_{(0-4\ h)}$ (ng × h/ml)	F (%)
IV formula[a](n = 2)	Single (2.5 mg/kg × 1)	4817.2	0.0	1.31	3693.0	100.0
IV formula[a](n = 4)	Single (5 mg/kg × 1)	6757.0 (408.0)	0.0 (0.0)	0.87 (0.18)	7889.8 (930.9)	100.0
IN formula[b](n = 2)	Single (2.5 mg/kg × 1)	1440.6	12.5	0.54	2671.7	72.3
IN formula[c](n = 3)	Single (5 mg/kg × 1)	2503.0 (191.5)	31.7 (12.6)	0.40 (0.02)	6233.2 (229.0)	79.0 (2.9)
IN formula[c] (n = 3)	Double[d] (5 mg/kg × 2)	4443.2 (893.5)	40.0 (8.7)	0.38 (0.04)	11674.6 (1903.3)	74.0[e] (12.1)

[a] IV formula: 1.5% MCA solution in 40% PEG 400–60% water.
[b] IN formula: 5% MCA solution in 1% SGC/60% PG–30% EtOH–10% water.
[c] IN formula: 10% MCA solution in 1% SGC/60% PG–30% EtOH–10% water.
[d] Interval between doses, 5 min.
[e] Normalized data determined using the following equation: $F = [(AUC_{IN,\ 5\ mg/kg,\ double})/(2 \times AUC_{IV,\ 5\ mg/kg,\ single})] \times 100$.
[f] Values given in parenthesis are the standard deviations for n = 3–4.

(Bechgaard et al., 1997) was employed as a simple and fast method of screening the absorption efficiency of many sample preparations; therefore the need to perform plasma analyses in the PK studies might be reduced.

The comparative results for the mean muscle relaxation times in the PD studies and the times to reach peak plasma levels in the PK studies determined after IV and IN administrations of DZ (1 mg/kg dose) and CZ (0.2 mg/kg dose) to rabbits are summarized in Table 5. The nasal administration of DZ and CZ formulations in 1% SGC containing EtOH/PG/water cosolvent elicited a very fast PD response, which was less than 1.5 min. In turn, this response time appeared to be essentially identical to that seen from an IV injection of a benzodiazepine. In addition, there was a correlation between the T_{max} and the time for induction of the pharmacological response after the IN administration of the DZ formulations. As shown in Table 5, IN formula 3 prepared with Cremophor EL as the vehicle showed a fairly long T_{max} (35 min). In the PD study, this formulation did not produce detectable muscle relaxation within 60 min after a single IN administration, but when re-sprayed 5 min after the first dosing, it showed a discernible muscle relaxing effect approximately 20 min after the second IN dosing. From these results, it appears that the pharmacological testing might be a simple and fast method for screening the absorption of IN formulations of benzodiazepines and other anticonvulsants possessing muscle relaxant effects.

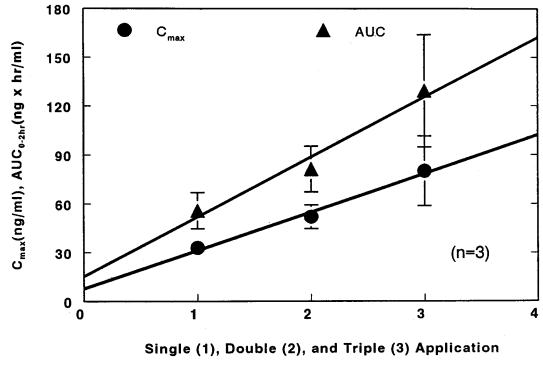

Fig. 5. Peak plasma levels and $AUC_{0-2\ h}$ obtained after single, double, and triple IN administration of CZ solutions at 0.2 mg/kg dose in rabbits.

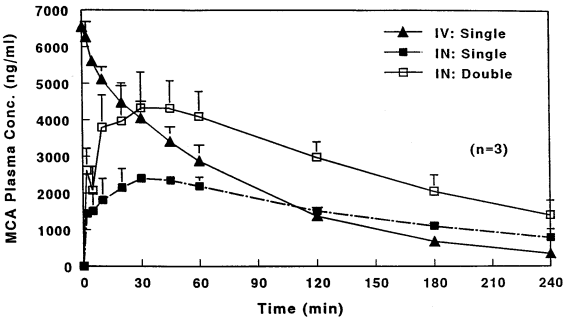

Fig. 6. Mean plasma concentration-time profiles of MCA after single IV and single and double IN administration of the drug solutions at 5 mg/kg dose in rabbits.

In conclusion, the results of the PK and PD studies suggest that single or repeated IN applications of DZ, CZ, and MCA formulated as solutions containing 1% SGC in a hydroalcohol–glycolic cosolvent system might be a viable approach to producing a rapid-onset delivery of the anticonvulsive agents. The peak plasma concentrations of DZ, which were observed within 2–3 min, could be modulated via a variation of the EtOH/PG volume ratio of the IN formulations. Through the IN application, the anticovulsants might be promptly and timely delivered to patients in need of emergency treatment for status epilepticus and other forms of seizures. In addition, the IN delivery systems of benzodiazepines might be utilized as an auxiliary sedation in surgical procedures. A further toxicological study should be performed to determine the therapeutic benefit to risk ratio of the rapid-onset IN drug delivery systems.

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Table 5
Comparison of the T_{max} and times of PD responses induced after the IV and IN administration of DZ and CZ in various formulations to rabbits

Drug	Formulation	Dosing (mg/kg)	T_{max} (min)	Time to induce PD response (min)
DZ	IV formula[a] ($n=3$)	Single (1 mg/kg × 1)	0.0	1.1 ± 0.2
	IN formula 2[b] ($n=3$)	Single (1 mg/kg × 1)	2.0	1.5 ± 0.5
	IN formula 3[c] ($n=3$)	Single (1 mg/kg × 1)	35.0	No response
	IN formula 3[c] ($n=3$)	Double[f] (1 mg/kg × 2)	na	19.8 ± 1.8
CZ	IV formula[d] ($n=3$)	Single (0.2 mg/kg × 1)	0.0	1.7 ± 0.5
	IN formula[e] ($n=3$)	Single[e] (0.2 mg/kg × 1)	2.0	1.4 ± 0.7

[a] DZ IV formula: 0.5% diazepam injection, USP, Elkins-Sinn, Inc.
[b] DZ IN formula 2: 2% diazepam solution in 1% SGC/60% PG–30% EtOH–10% water.
[c] DZ IN formula 3: 2% diazepam solution in Cremophor EL.
[d] CZ IV formula: 0.15% clonazepam solution in 40% PG–30% EtOH–30% water.
[e] CZ IN formula: 0.42% clonazepam solution in 1% SGC/60% PG-30% EtOH–10% water.
[f] Application time interval for repeated dosing: 5 min. na, Not available.

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