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Development of an ethyl laurate-based microemulsion for rapid-onset intranasal delivery of diazepam

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Abstract

An ethyl laurate-based microemulsion system with Tween 80 as surfactant, propylene glycol and ethanol as cosolvents was developed for intranasal delivery of diazepam. Phase behavior and solubilization capacity of the microemulsion system were characterized and in vivo nasal absorption of diazepam from microemulsion formulations was investigated in rabbits. A single isotropic region, which is considered as a bicontinuous microemulsion, was found in the pseudo-ternary phase diagrams developed at various Tween 80: propylene glycol: ethanol ratios. With the increase of Tween 80 concentration, the microemulsion region area, microemulsion viscosity, and the amount of H_2O and ethyl laurate solubilized into the microemulsion system increased; however, the increase of ethanol percentage produced opposite effects. Diazepam, a practically water-insoluble drug, displayed a high solubility of 41 mg/ml in a microemulsion consisting of 15% ethyl laurate, 15% H_2O, and 70% (w/w) surfactant/cosurfactant (Tween 80:propylene glycol:ethanol at 1:1:1 weight ratio). Nasal absorption of diazepam from this microemulsion was found to be fairly rapid. At 2 mg/kg dose, the maximum drug plasma concentration was arrived within 2–3 min, and the bioavailability (0–2 h) after nasal spray compared with intravenous injection was about 50%. These results suggest that this ethyl laurate-based microemulsion may be a useful approach for the rapid-onset delivery of diazepam during the emergency treatment of status epilepticus.

Keywords: Diazepam; Microemulsion; Solubilization; Intranasal absorption; Status epilepticus

1. Introduction

Status epilepticus is a serious neurological emergency. The goal of treatment is rapid termination of seizure activity because the longer the episode of status epilepticus is untreated, the more difficult it is to control and the greater the risk of permanent brain damage (McNamara, 1996). Currently, intravenous (IV) administration of diazepam (DZ) is well accepted as one of the choices for the initial treatment of status epilepticus while the underlying causes are being treated and a long-acting anticonvulsant is being administered.

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Although IV administration is probably the most rapid way of seizure suppression, an alternative and more convenient approach is highly needed when IV administration is not immediately available, for instance, because of the delay on transferring patient to hospital or arriving of emergency medical personnel. Systemic drug delivery through nasal route has been demonstrated as a possible alternative to IV injection and a promising approach for rapid-onset delivery of CNS medications, owing to the extensive microcirculation underneath nasal mucosa and the preferential drug delivery to brain (Kumar et al., 1974; Sakane et al., 1991).

For nasal delivery of DZ, two challenges exist in formulation development. One is the requirement of DZ solubilization. The aqueous solubility of DZ is < 50 μg/ml. Since the therapeutic dose of DZ for the treatment of status epilepticus is 5–10 mg and the effective nasal delivery volume is ≤ 300 μl (150 μl/nostril), the target concentration of DZ in nasal formulation is 17–34 mg/ml. The other challenge is the achievement of rapid-onset nasal absorption of DZ to meet the emergency therapeutic purpose of this formulation.

The development strategies on DZ nasal delivery have been focusing on the solubilization of practically insoluble DZ using DZ highly soluble solvent vehicles such as Cremophor EL (Lau and Slattery, 1989), glycofurol, tetraethyleneglycol (Bechgaard et al., 1997a,b), and cosolvent of propylene glycol, ethanol and H_2O (Li et al., 2000). In our previous studies (Li et al., 2000), an aqueous ternary cosolvent of 60% propylene glycol, 30% ethanol and 10% H_2O provided desired solubility of DZ (21 mg/ml). After nasal administration, this cosolvent produced a rapid in vivo absorption ($t_{max} = 4$ min) and a bioavailability of 59%, compared with IV. The addition of 1% sodium glycocholate further increased the bioavailability to 77% with a t_{max} of 2 min.

Microemulsions, optically isotropic and thermodynamically stable systems of water, oil, surfactant and cosurfactant, have been studied as drug delivery systems on account of their solubilization capacity for poorly water-soluble drugs as well as their enhancement effect on topical and systemic drug availability. For instance, oral microemulsion formulations have been successfully developed for cyclosporine, a highly lipophilic and poorly aqueous-soluble drug, to improve its oral bioavailability and to reduce the absorption variation (Ritschel et al., 1990; Kim et al., 1996). Microemulsions have also been considered as topical (Linn, 1990; Garcia-Celma et al., 1994), transdermal (Thevenin et al., 1996), and parenteral (Corswant et al., 1998) drug delivery systems. In terms of nasal delivery, very few studies have reported the use of microemulsion as the delivery system, although the use of emulsion has been seen (Kararli et al., 1992).

The aim of this current project was to develop a microemulsion system using GRAS (generally regarded as safe) materials for the solubilization and rapid-onset intranasal delivery of diazepam. The solution-like feature of microemulsion could provide advantages over regular emulsion in terms of the sprayability, dose uniformity and formulation physical stability.

2. Materials and methods

2.1. Materials

DZ and clonazepam (analytical internal standard) were purchased from Sigma (St. Louis, MO). DZ injection was obtained from Elkins-Sinn (Cherry Hill, NJ). Ethyl laurate, ethyl oleate, Tween 80, propylene glycol, phosphoric acid, and ethylenediaminetetracetic acid (EDTA) were purchased from Sigma (St. Louis, MO). Other chemicals are HPLC or analytical grade and used as received. Water was deionized and distilled in the laboratory.

2.2. HPLC assay methods for in vitro and plasma samples

A HPLC system equipped with 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) was used for DZ concentration determination. The column was a reversed phase Symmetry C_{18} column (15 cm ×

3.9 mm I.D. × 5 μm; Waters Corporation) used at room temperature.

For solubility and stability samples, the mobile phase was a mixture of methanol:H_2O at 70:30 (v/v) at a flow rate of 1.0 ml/min. DZ was detected at 254 nm with a retention time of 4.1 min.

For plasma samples, the mobile phase was 50% methanol/10% acetonitrile/40% (pH 3.5) 0.03 M KH_2PO_4/H_3PO_4 buffer (v/v) at a flow rate of 1.0 ml/min and a detection wavelength of 229 nm. The retention time was 8.6 min for DZ and 4.0 min for the analytical internal standard, clonazepam.

2.3. Solubility determination

Drug powder of DZ was added in excess to ethyl laurate (oil), ethyl oleate (oil), Tween 80 (surfactant), propylene glycol (cosurfactant), ethanol (cosurfactant), and H_2O, respectively. After shaking at 25 °C for 24 h, samples were centrifuged, and the supernatant was properly diluted with methanol and injected into HPLC for DZ concentration determination.

2.4. Phase diagram preparation and microemulsion formulation

The pseudo-ternary phase diagrams of oil, surfactant/cosurfactant, and H_2O were developed using H_2O titration method: the mixtures of oil and surfactant/cosurfactant at certain weight ratios were diluted with H_2O in a drop-wise manner. Five phase diagrams were prepared with Tween 80:propylene glycol:ethanol weight ratios defined at 3:1:0.25, 1:3:0.25, 1:1:0.25, 1:1:0.5, and 1:1:1. For each phase diagram at specific surfactant/cosurfactant ratio, ten transparent and homogenous mixtures of oil: (Tween 80:propylene glycol:ethanol) at 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 (w/w) were formed under the mixing of a magnetic stirrer. Then, each mixture was titrated with H_2O and visually observed for phase clarity and flowability. Gels were claimed for those mixtures that did not show a change in the meniscus after tilted to an angle of 90°. No heating was conducted during the preparation; however, well-covered magnetic stirring was performed throughout the titration process for a thorough mixing.

After the identification of microemulsion region in the phase diagrams, the microemulsion formulations were selected at desired component ratios. The preparation of selected microemulsions was simply performed by adding the weighed components together and stirring to form a clear microemulsion. The DZ-incorporating microemulsions were prepared by dissolving the drug powder into the microemulsion systems. In order to confirm the microemulsion formation, the selected microemulsions were characterized as follows.

2.5. Characterization of microemulsion

The solubilization capacity of selected microemulsion systems for DZ was investigated by adding an excess amount of drug powder to microemulsions. After being shaken for 24 h, the mixture was centrifuged and HPLC-analyzed.

The particle size analysis of selected microemulsions was conducted using a dynamic light scattering method with a Nicomp 380-Submicron Particle Sizer (Particle Sizing Systems, Santa Barbara, CA). The wavelength used was 632.8 nm.

The physical and chemical stability of plain and DZ-incorporating microemulsions were studied via clarity and phase separation observation, particle size determination, and HPLC analysis of DZ degradation at room temperature for up to 2 months.

The electric conductivity of microemulsions was measured with a VWR digital conductivity meter (Traceable™) equipped with a platinum electrode with a cell constant of 1.0. Calibration of the electrode was conducted before sample determination.

2.6. Nasal absorption studies

2.6.1. Experimental procedure

New Zealand white rabbits (3.0–4.0 kg), obtained from Marland Breeding Farms (Hewitt, NJ), were used for DZ nasal and IV administration with a washout period of 2 weeks.

Rabbits were weighed and restrained in rabbit restrainers before the experiment. For IV administration, an IV infusion of DZ injection (5 mg/ml) was delivered through the marginal ear vein of

rabbit at 1 mg/kg dose for over 20 s. For intranasal administration, 100 μl of the nasal formulation (20 or 40 mg/ml DZ in microemulsions) was sprayed into each nostril of rabbit using a metered-dose pump spray device (Pfeiffer, Princeton, NJ) for within 5 s. Blood sampling (0.5 ml) started before dosing and at 2, 5, 10, 20, 30, 45, 60, and 120 min after dosing via an artery catheter set up at the rabbit ear. Blood samples were anticoagulated with EDTA and centrifuged at 3000 rpm for 15 min. Plasma was separated and stored at −20 °C until analysis.

2.6.2. Plasma treatment

Plasma DZ concentrations were analyzed by HPLC after precipitating the plasma samples with 0.01% (v/v) perchloric acid in acetonitrile. The above precipitation solution (250 μl) containing clonazepam 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 the HPLC. Under these analytical conditions, the detection limit for DZ was found to be 15 ng/ml.

2.6.3. Calculation and statistics

All plasma concentration data were dose- and weight-normalized, and then analyzed using WinNonlin noncompartmental model (Scientific Consulting, Apex, NC). The area-under-the curve (AUC_{0-t}) was determined by the linear trapezoidal method. The C_{max} following IV injection was estimated by extrapolating the initial plasma drug concentration–time curve to the Y-axis at time zero (C_0), and the C_{max}, C_{2min}, and t_{max} values of the nasal administration were read directly from the concentration-time profile. The $t_{1/2}$ was calculated by fitting the data of the terminal portion of the pharmacokinetic profile by a log-linear regression equation. The absolute bioavailability (F%) of nasal administration from microemulsions was calculated using the following Eq. (1):

$$F\ (\%) = (AUC_{0-t,\,nasal} \times Dose_{IV})/(AUC_{0-t,\,IV} \times Dose_{nasal}) \times 100 \quad (1)$$

Statistical analysis was performed utilizing standard method; Student's t-test was employed for calculating the significance ($P < 0.05$).

Table 1
Solubility of DZ in various microemulsion components at 25 °C

Component	DZ (mg/ml)
Ethyl laurate	39.6 ± 2.0
Ethyl oleate	14.9 ± 0.3
Tween 80	43.3 ± 4.5
Propylene glycol	16.7 ± 0.6
Ethanol	41.4 ± 2.8
H_2O	0.04 ± 0.01

Values are mean ± S.D. for $n = 3$.

3. Results and discussion

3.1. DZ solubility in microemulsion components

The solubility of DZ in individual oil, surfactant, and cosurfactant comprising the microemulsion system was studied and is displayed in Table 1. Two types of oil were evaluated, ethyl laurate and ethyl oleate. As observed from the table, the solubility of DZ in ethyl laurate was about 40 mg/ml, much higher than its solubility in ethyl oleate (about 15 mg/ml). Also, ethyl laurate is chemically more stable than ethyl oleate (unsaturated fatty acid chain). Therefore, ethyl laurate was selected as the oil phase for the microemulsion development.

3.2. Phase behavior

The pseudo-ternary phase diagrams with various Tween 80:propylene glycol:ethanol weight ratios are displayed in Figs. 1 and 2. The translucent and low viscosity microemulsion area is presented in the phase diagrams as ME area. No distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) microemulsions was seen; therefore, this single isotropic region is considered as a bicontinuous microemulsion. The gel area indi-

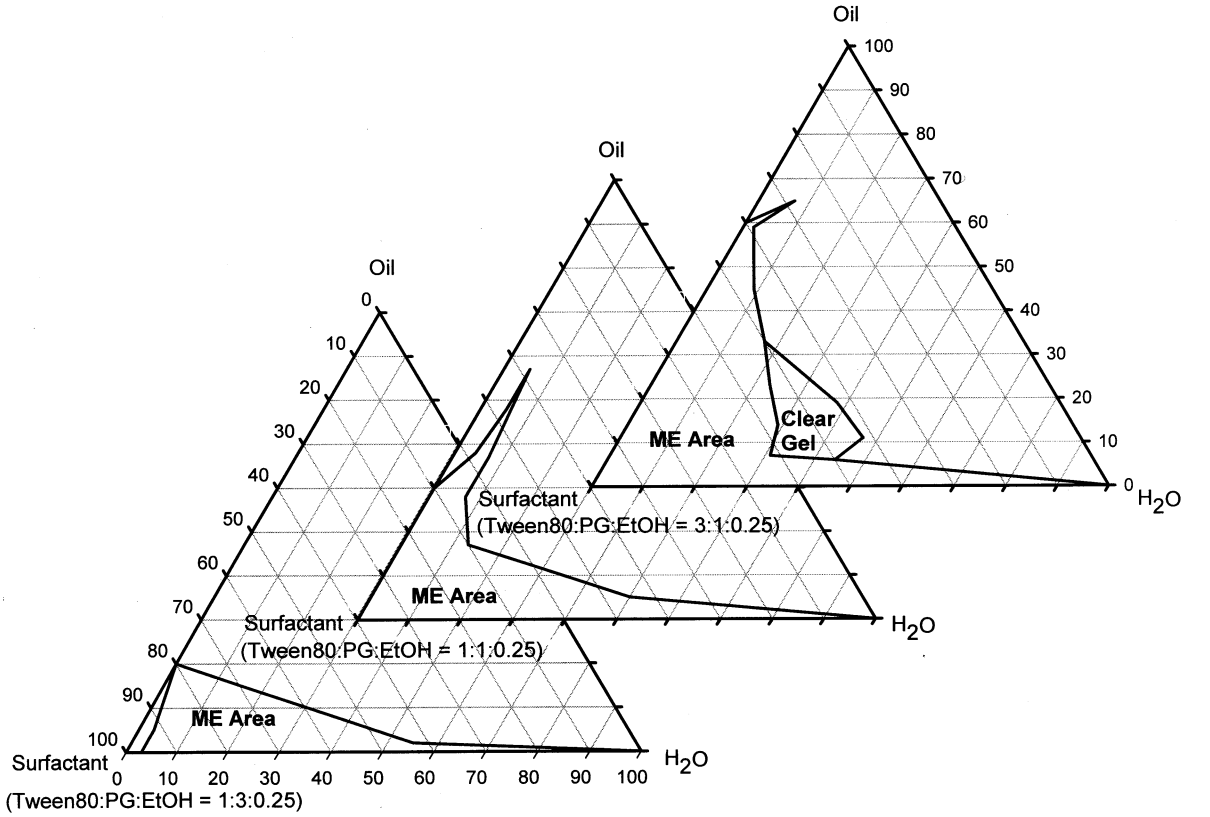

Fig. 1. Phase diagrams of oil-surfactant-H_2O system at different Tween 80:propylene glycol:ethanol ratios of 1:3:0.25, 1:1:0.25, and 3:1:0.25: Influence of Tween 80 concentration.

cates the clear and high viscosity region. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual identification. From these phase diagrams, the influence of relative surfactant:cosurfactant concentrations on the microemulsion isotropic region can be evidently seen. Fig. 1 shows the effect of Tween 80:propylene glycol ratio on the phase behavior of these pseudo-ternary systems. While keeping ethanol percentage constant, and changing Tween 80:propylene glycol ratio, such as Tween 80:propylene glycol:ethanol = 1:3:0.25, 1:1:0.25, and 3:1:0.25, the microemulsion region increased in size with the higher surfactant concentration. This increase was toward the oil–H_2O axis, indicating that by increasing the Tween 80 concentration, the maximum amount of H_2O and ethyl laurate that could be solubilized into the microemulsion increased. Fig. 2 shows the influence of ethanol concentration on the microemulsion region. Comparison of three phase diagrams at Tween 80:propylene glycol:ethanol weight ratios of 1:1:0.25, 1:1:0.5, and 1:1:1 revealed that the presence of more ethanol reduced the H_2O incorporation capacity. As seen from the phase diagrams, the isotropic region decreased in size with the increase of ethanol weight ratio, while the surfactant:cosurfactant ratio remained the same.

The thickness of microemulsion was also affected by the surfactant and ethanol content. With the higher weight percentage of Tween 80, the thickness of the microemulsion formulation increased, and a gel area was seen in the 3:1:0.25 phase diagram. On the other hand, with the decrease in ethanol percentage, the thickness of the microemulsion decreased. For nasal delivery, a

less viscous microemulsion is preferred considering the requirement of sprayability of nasal formulation by the pump device and the dispersion uniformity of the spray.

3.3. Microemulsion composition and characterization

In this study, three criteria were defined for the selection of microemulsion formulations from the developed phase diagrams: (1) DZ should have a good solubility in the selected microemulsion; (2) The microemulsion formulation should be sprayable using a pump spray device in order to obtain a better distribution and larger surface area with small droplets of spray; (3) The H_2O percentage should be more than 10%. Our previous irritation evaluation using scanning electron microscopy showed that incorporation of H_2O tended to reduce the irritation caused by a cosolvent nasal formulation consisting of 60% propylene glycol–30% ethanol–10% H_2O (Li et al., 2000). Hence, 10% of H_2O was defined as the minimal H_2O content of the selected microemulsion formulation. Two microemulsions were selected from the 1:1:0.25 and 1:1:1 phase diagrams and defined as ME1 and ME2. The composition of these MEs is shown in Table 2.

Table 3 shows the characterization of these two MEs in terms of solubilization capacity, particle size distribution, and conductivity. The solubility of DZ, a practically water-insoluble compound (40 μg/ml), was improved dramatically by the microemulsions, and ME2 (41 mg/ml) produced a higher solubilizing capacity of DZ than ME1 (28 mg/ml). Compared with the DZ solubility (21

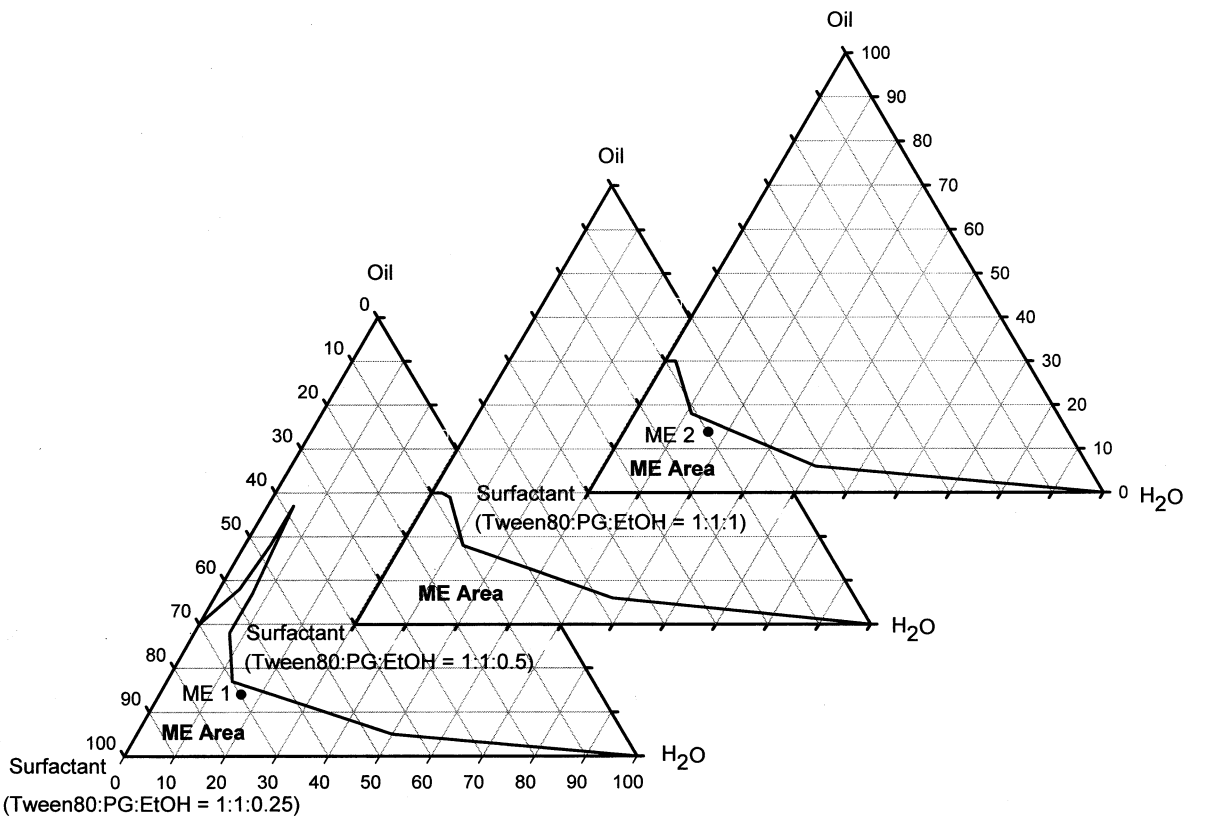

Fig. 2. Phase diagrams of oil-surfactant-H_2O system at different Tween 80:propylene glycol:ethanol ratios of 1:1:0.25, 1:1:0.5, and 1:1:1: Influence of ethanol concentration.

Table 2
Composition of the selected microemulsion formulations

Component	ME1: (Tween 80:propylene glycol:ethanol = 1:1:0.25) (% w/w)	ME2: (Tween 80:propylene glycol:ethanol = 1:1:1) (% w/w)
Ethyl laurate	15.0	15.0
Tween 80	31.0	23.3
Propylene glycol	31.0	23.3
Ethanol	8.0	23.3
H_2O	15.0	15.0

Table 3
Physico-chemical characterization of microemulsion formulations

Microemulsion	Solubilization[a] (mg/ml)	Particle size distribution[b] (nm)	Conductivity (μΩ/cm)
ME1	28.1 ± 0.7	66 ± 68	13
ME2	41.0 ± 0.5	67 ± 27	20

[a] Solubility (mean ± S.D., $n = 3$) of diazepam in microemulsion formulation at RT.
[b] Particle size (mean ± S.D.) of the dispersed phase of microemulsion.

mg/ml) in the cosolvent consisting 60% propylene glycol–30% ethanol–10% water, ME2 showed an approximately two times greater solubilization capacity than the cosolvent, which could provide more formulation flexibility for nasal delivery of DZ to reach its therapeutic IV dose of 5–10 mg.

The particle size of ME1 and ME2 fell into the size range of microemulsion (10–150 nm) as shown in Table 3. Although the mean particle sizes of these two MEs were about the same, with a mean diameter of 66 nm for ME1 and 67 nm for ME2, the size distribution of ME2 was much narrower than ME1. For instance, the particle size standard deviation for ME2 was 27 nm, whereas it was 68 nm for ME1. Therefore, ME2 was deemed to contain more uniformly sized droplets than ME1.

These two microemulsion formulations were physically stable at room temperature with the presence or absence of DZ for a period of 2 months, without the occurrence of phase separation and significant particle size change. No degradation of DZ was detected during the study period.

The electric conductivity values for ME1 and ME2 were 13 and 20 μΩ/cm, respectively. The type of these MEs could not be distinctly defined as w/o or o/w microemulsion according to these conductivity values; however, the conductivity appears to rise with the increase of ethanol percentage.

3.4. Nasal absorption of DZ

In vivo absorption of DZ following nasal administration of ME1 and ME2 were evaluated in rabbits, compared with IV administration. Fig. 3

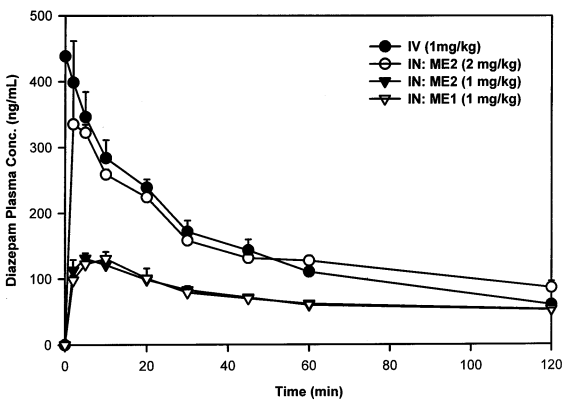

Fig. 3. Mean plasma concentration – time profiles of diazepam after IV and nasal administration of microemulsions at 1 or 2 mg/kg dose in rabbits.

Table 4
Bioavailability and pharmacokinetic parameters of DZ after IV and nasal administration from microemulsion formulations in rabbits

Route/ME	Dose (mg/kg)	C_{2min}[a] (ng/ml)	C_{max} (ng/ml)	t_{max} (min)	$t_{1/2}$ (h)	AUC_{0-2h} (ng × h/ml)	F[b] (%)
IV	1	398.8 ± 63.0	438.6 ± 84.7	0.0 ± 0.0	1.03 ± 0.33	293.1 ± 7.0	100.0
IN/ME1	1	97.9 ± 7.5	130.6 ± 10.6	10.0 ± 0.0	2.85 ± 0.50	144.4 ± 1.79	49.3 ± 0.6
IN/ME2	1	113.1 ± 15.7	133.6 ± 3.8	6.7 ± 2.9	2.55 ± 0.70	143.7 ± 9.5	49.0 ± 3.2
IN/ME2	2	335.3 ± 68.1	352.3 ± 40.8	3.0 ± 1.7	2.02 ± 0.75	293.8 ± 8.62	50.1 ± 2.9

IV: 0.5% diazepam injection, USP, Elkins-Sinn. IN: Intranasal. 1 mg/kg: 20 mg/ml DZ in microemulsion. 2 mg/kg: 40 mg/ml DZ in microemulsion. $n = 3$ for all experiments. Values are mean ± S.D.

[a] Diazepam plasma concentration at 2 min.

[b] $F\% = (AUC_{nasal} \times Dose_{IV})/(AUC_{IV} \times Dose_{nasal}) \times 100$.

represents the mean plasma concentration–time profiles of DZ after IV and nasal administration of these two MEs at 1 mg/kg dose and ME2 at 2 mg/kg dose. The corresponding bioavailability and pharmacokinetic parameters are shown in Table 4. At 1 mg/kg dose, the nasal delivery of ME1 and ME2 produced similar bioavailability of 49% relative to IV administration. However, ME2 showed a trend of faster DZ absorption than ME1. The mean peak time of DZ absorption from ME2 was 6.7 min, more rapid than the 10.0 min from ME1. In addition, the plasma level at 2 min (C_{2min}) of ME2 (113.1 ng/ml) was slightly greater than ME1 (97.9 ng/ml). This difference might be explained by the higher percentage of ethanol presented in ME2.

Another advantage of ME2 over ME1 is its greater solubility enhancing effect on DZ. As mentioned before, ME2 increased DZ solubility to 41 mg/ml; therefore, at the same nasal spray volume (100 μl to each nostril), this formulation was able to load DZ to a doubled dose of 2 mg/kg. As shown in Table 4 and Fig. 3, the intranasal bioavailability ($\sim 50\%$) of DZ from ME2 was dose-independent. AUC_{0-2h} at 2 mg/kg dose (40 mg/ml DZ in microemulsion) was approximately 2-fold greater than 1 mg/kg dose (20 mg/ml DZ in microemulsion). DZ was absorbed very rapidly from 40 mg/ml DZ in ME2 with the peak time of 2.0–3.0 min. The C_{2min} and C_{max} at 2 mg/kg dose were about 2.5–3.0 times higher than those at 1 mg/kg dose. The C_{2min} and AUC_{0-2h} of ME2 at 2 mg/kg dose reached about 84 and 100% of IV administration at 1 mg/kg. These results indicate that ME2 formulation, 15% ethyl laurate/23.3% Tween 80/23.3% propylene glycol/23.3% ethanol/15% H_2O, could be a promising approach for the acute intranasal delivery of DZ.

Considering the solubilization property, particle size analysis, and in vivo absorption findings, ME2 is believed to be a better formulation than ME1 for the rapid-onset intranasal delivery of DZ.

4. Conclusion

The microemulsion system comprising ethyl laurate, Tween 80, propylene glycol, ethanol and H_2O showed high solubilization capacity of DZ. At Tween 80:propylene glycol:ethanol weight ratio of 1:1:1, the microemulsion area appeared to be a bicontinuous system with less viscosity and good sprayability. In vivo absorption studies revealed that DZ absorption from a microemulsion of 15% ethyl laurate–23.3% Tween 80–23.3% propylene glycol–23.3% ethanol–15% H_2O at 2 mg/kg dose had a fairly rapid-onset of 2–3 min and a bioavailability of 50%. With a 50% bioavailability, a dose of 10–20 mg DZ is needed in order to produce the same effect as the IV formulation on status epilepticus. At a concentration of 40 mg/ml, 250–500 μl of the ME2 formulation is necessary for the nasal administration, which equals to 1–2 sprays per nostril.

In conclusion, the ethyl laurate-based microemulsion system might be a promising approach

for the rapid-onset intranasal delivery of DZ for the acute treatment of status epilepticus and other types of seizures. Further local and systemic toxicity evaluation of this microemulsion system is necessary in order to determine the therapeutic benefit/risk ratio.

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References

Bechgaard, E., Gizurarson, S., Hjortkjaer, R.K., 1997a. Solubilization of various benzodiazepines for intranasal administration, a pilot study. Pharm. Dev. Tech. 2, 293–296.

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

Corswant, C.V., Thoren, P., Engstrom, S., 1998. Triglyceride-based microemulsion for intravenous administration of sparingly soluble substances. J. Pharm. Sci. 87, 200–208.

Garcia-Celma, M.J., Azemar, N., Pes, M.A., Solans, C., 1994. Solubilization of antifungal drugs in water/POE (20) sorbitan monoleate/oil systems. Int. J. Pharm. 105, 77–81.

Kararli, T.T., Needham, T.E., Schoenhard, G., Baron, D.A., Schmidt, R.E., Katz, B., Belonio, B., 1992. Enhancement of nasal delivery of a renin inhibitor in the rat using emulsion formulations. Pharm. Res. 8, 1024–1028.

Kim, C.K., Ryuu, S.A., Park, K.M., Lim, S.L., Hwang, S.J., 1996. Preparation and physiochemical characterization of phase inverted water/oil microemulsion containing cyclosporin A. Int. J. Pharm. 147, 131–134.

Kumar, A., David, T.C., Saini, K.D., 1974. Uptake of radioactivity by body fluids and tissues in rhesus monkeys after intravenous injection or intranasal spray of tritium-labeled oestradiol and progesterone. Curr. Sci. 43, 435.

Lau, S.W.L., Slattery, J.T., 1989. Absorption of diazepam and lorazepam following intranasal administration. Int. J. Pharm. 54, 171–174.

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

Linn, E.E., 1990. Microemulsion for intradermal delivery of cetyl alcohol and octyl dimethyl PABA. Drug Dev. Ind. Pharm. 16, 899–920.

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. 478–485.

Ritschel, W.A., Adolph, S., Ritschel, G.B., Schroeder, T., 1990. Improvement of peroral absorption of cyclosporin A by microemulsions. Meth. Find. Exp. Clin. Pharmacol. 12, 127–134.

Sakane, T., Akizuki, M., Yoshida, M., Yamashita, S., Nadai, T., Hashida, M., Sezaki, H., 1991. Transport of cephalexin to the CSF directly from the nasal cavity. J. Pharm. Pharmacol. 43, 449–451.

Thevenin, M.A., Grossiord, J.L., Poelman, M.C., 1996. Sucrose esters/cosurfactant microemulsion systems for transdermal delivery: assessment of bicontinuous structures. Int. J. Pharm. 137, 177–186.