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RESEARCH ARTICLE

Semi-solid dosage form of clonazepam for rapid oral mucosal absorption

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Abstract

Background: In order to obtain an alternative to the intravenous (i.v.) dosage form of clonazepam (CZ), an oral droplet formulation of CZ was developed previously; however, the droplet was physically unstable. Therefore, in the present study, it was attempted to develop an easily-handled dosage form, which was more physically stable and allowed rapid drug absorption from oral mucosa.

Method: A semi-solid dosage form, composed of polyethylene glycol 1500 (PEG), CZ, and oleic acid (OA) at 37/1/2 (w/w) and named PEG/CZ/OA, and a semi-solid dosage form containing PEG and CZ at 39/1 (w/w), called PEG/CZ, were prepared. Their physical stability in air at room temperature and oral mucosal absorption in rats were investigated.

Results: The semi-solid dosage forms were much more stable physically than the droplet, that is, no recrystallization of CZ was observed for at least 8 days. The effective concentration for humans and rats (20 ng/mL or more) was achieved within 30 min after buccal administration for both PEG/CZ/OA and PEG/CZ. The plasma concentration increased gradually and less varied at each time point for PEG/CZ/OA. PEG/CZ/OA was found to show more rapid and higher absorption of CZ in buccal administration than in sublingual administration.

Conclusion: Buccal administration with the semi-solid dosage PEG/CZ with or without OA was suggested to be a possibly useful novel dosage form as an alternative to i.v. injection.

Keywords: Clonazepam, oral mucosal absorption, semi-solid dosage form, physical stability, buccal administration

Introduction

Benzodiazepine drugs are generally used for the suppression of epileptic seizure, sedation, or antianxiety[1]. Epileptic convulsion is a neurological emergency, and prompt treatment is essential to reduce seizures and damage[2]. Intravenous (i.v.) injection of benzodiazepines is the most effective method to suppress epileptic seizures, because it can supply an effective concentration quickly; however, as sterile equipment and skilled personnel are required for i.v. injection, other administration routes have been studied as alternatives. Diazepam (DZ) is often used for the treatment of epileptic convulsion, and rectal and nasal administrations have been studied as alternatives to i.v. injection[3]; however, after their administration, several– some dozens of minutes are necessary to achieve effective plasma levels[4]. Previously, we developed a DZ oral patch, and achieved quick absorption within 10 min to reach an effective concentration, though maintenance of the plasma level was not very good[5]. For DZ, a plasma concentration of more than several 100 μg/mL is needed to treat epileptic convulsions[6]. On the other hand, clonazepam (CZ) has therapeutic effects against various epileptic convulsions, and its effective concentration is much lower than that of DZ[6,7]. As in the case of DZ, CZ formulations alternative to i.v. injection, such as rectal, nasal, or buccal, have been studied to enable its use during emergencies[8–10]. We developed a propylene glycol (PG) droplet containing CZ and oleic acid (OA) as a formulation for oral mucosa absorption, with which the effective concentration, that is, a plasma concentration of more than 20 ng/mL, was achieved within 10 min after administration[11]. However,

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CZ dissolved in the PG droplet was observed to recrystallize within 1 day because of its supersaturation. This poor physical stability was a defect of this droplet formulation. In order to solve this problem, semi-solid dosage forms containing CZ were developed using polyethylene glycol 1500 (PEG) as a matrix base. In addition, OA, often used as an absorption enhancer in mucosal absorption[5,11–13], was applied to the dosage form. That is, since OA generally enhances the absorption of lipophilic or non-polar drugs by the promotion of fluidity of the lipid membrane[13,14], it was used as an additive in the preparation of the dosage form containing a lipophilic drug CZ. The dosage forms with and without OA were developed, and examined for physical stability and oral mucosal absorption.

Materials and methods

Materials

CZ, PG, and urethane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). PEG was obtained from Nikko Pharmaceutical Co., Ltd. (Hashima, Japan). OA was supplied by NOF Corporation (Tokyo, Japan). All other chemicals used were of reagent grade.

Animals

Male Wistar rats (7 weeks old, 250 g) were purchased from Tokyo Laboratory Animal Science Co., Ltd., and used soon for *in vivo* experiments. The animals were kept on the breeding diet MF supplied by Oriental Yeast Co. Ltd. (Tokyo, Japan) with water *ad libitum* in a room where the temperature and relative humidity were kept at 23 ± 1°C and 60 ± 1%, respectively. The light-dark cycle was set at 12 h. The experimental protocol was approved by the Committee on Animal Research of Hoshi University (Tokyo, Japan), and animal experiments were performed according to the Guiding Principles for the Care and Use of Laboratory Animals of Hoshi University.

Preparation of formulations

Physical mixtures, semi-solid dosage forms, and semi-solid matrix base were prepared as follows (Table 1). (i) Physical mixture of PEG and CZ (PEG/CZ-PM): PEG (975 mg) and CZ (25 mg) were mixed sufficiently on a Teflon plate using a spatula. (ii) Physical mixture of PEG, CZ, and OA (PEG/CZ/OA-PM): PEG (925 mg), OA (50 mg), and CZ (25 mg) were mixed sufficiently on a Teflon plate using a spatula. (iii) Semi-solid dosage form of PEG and CZ (PEG/CZ-SS): PEG (975 mg) and CZ (25 mg) were mixed in a glass tube, heated at 80°C for 1.5 min to dissolve completely, and mixed with a vortex mixer. Vortex mixing was continued at room temperature until the mixed solution became semi-solid. The resultant semi-solid was used as PEG/CZ-SS. (iv) Semi-solid dosage form of PEG, CZ, and OA (PEG/CZ/OA-SS): PEG (925 mg) and CZ (25 mg) were mixed in a glass tube, heated at 80°C for 1.5 min to dissolved completely, and mixed with a vortex mixer. Vortex mixing was continued at room temperature until the mixed solution became semi-solid. OA (50 mg) was added, and heated at 70°C to dissolve the mixture completely. The solution was mixed with a vortex mixer at room temperature until the solution mixture became semi-solid. The resultant semi-solid was used as PEG/CZ/OA-SS. (v) Semi-solid base made of PEG and OA (PEG/OA-SS): after PEG (925 mg) had been dissolved in a glass tube at 80°C, it was cooled to room temperature. OA (50 mg) was added and the mixture was heated at 70°C to dissolve completely by vortex mixing. Mixing was continued at room temperature until the solution got to be semi-solid.

Table 1. Formulations for physical state studies.

Formulation	PEG (mg)	CZ (mg)	OA (mg)	Total (mg)
PEG/CZ-PM	975	25	—	1000
PEG/CZ/OA-PM	925	25	50	1000
PEG/CZ-SS	975	25	—	1000
PEG/CZ/OA-SS	925	25	50	1000
PEG/OA-SS	925	—	50	975

PEG/CZ-PM, physical mixture of PEG and CZ; PEG/CZ/OA-PM, physical mixture of PEG, CZ and OA; PEG/CZ-SS, semi-solid dosage form of PEG and CZ; PEG/CZ/OA-SS, semi-solid dosage form of PEG, CZ and OA; PEG/OA-SS, semi-solid dosage form of PEG and OA.
CZ, clonazepam; OA, oleic acid; PEG, polyethylene glycol 1500.

Droplet formulations were prepared according to the previous study[11]. (i) Droplet containing PG and CZ (PG/CZ-D): CZ (5 mg) was added to PG (195 mg), heated at 90°C and shaken vigorously until complete dissolution. The solution was left at room temperature and used as PG/CZ-D. (ii) Droplet containing PG, CZ, and OA (PG/CZ/OA-D): CZ (5 mg) was added to PG (185 mg), heated at 90°C and shaken vigorously until complete dissolution. The solution was cooled to room temperature, and OA (10 mg) was added. The mixture was heated at 90°C to dissolve completely. The solution was left at room temperature, and used as PG/CZ/OA-D.

Examination of physical state and physical stability

Physical states of semi-solid dosage forms were investigated by X-ray diffraction and microscopic observation. Each preparation containing 3 mg CZ was placed on the sample plate, and underwent X-ray diffraction using a powder X-ray diffractometer RINT1400 (Rigaku Corporation, Tokyo, Japan), in which Cu Kα radiation was used at 50 kV and 100 mA with a scan speed of 4°/min and a scan range of 5–60°. For microscopic observation, the sample was observed using a regular optical microscope, with which the physical stability of semi-solid dosage forms was examined for recrystallization of CZ in the matrix.

In vivo absorption studies

The composition of each administration sample is shown in Table 2. Namely, 40 mg of sample containing 1 mg CZ was administered per rat. The animal experiment was performed as follows. Rats were anesthetized by i.p. injection of 1.2 mL of a 25% (w/v) urethane solution in

saline. After blood (0.3 mL) was withdrawn as a blank, the semi-solid dosage form (40 mg) on the oblate (6 × 6 mm) was administered to each rat buccally in the oral cavity between the lower gum and bottom lip, or sublingually. Blood samples (0.3 mL) were withdrawn via the jugular vein at 10, 15, 30, and 60 min after administration. Plasma was separated by centrifugation of the blood at 1500×*g* for 10 min. Plasma (0.1 mL) was mixed with 0.2 mL of 0.1 M NaOH aqueous solution and stirred vigorously. Then, 4 mL of the hexane/ethyl acetate (9:1, v/v) mixture was added, shaken vigorously and centrifuged at 1500×*g* for 10 min. The resultant organic phase (3.6 mL) was taken, and the solvent was evaporated under nitrogen gas. The residue was dissolved in 40 µL high-performance liquid chromatography (HPLC) mobile phase, and 20 µL of the solution was injected onto the HPLC column to determine the CZ concentration.

For statistical analysis, the unpaired *t*-test was used for comparison of two groups, and analysis of variance followed by the Scheffe post-hoc test was applied for comparison of more than two groups. Significant difference was set as $P < 0.05$.

Table 2. Compositions of the formulation samples (40 mg) used in animal studies.

Formulation	PEG (mg)	CZ (mg)	OA (mg)
PEG/CZ-SS	39	1	—
PEG/CZ/OA-SS	37	1	2

PEG/CZ-SS, semi-solid dosage form of PEG and CZ; PEG/CZ/OA-SS, semi-solid dosage form of PEG, CZ and OA.
CZ, clonazepam; OA, oleic acid; PEG, polyethylene glycol 1500.

HPLC assay

The concentration of CZ in the sample was determined by HPLC at room temperature according to the previous paper[11]. Briefly, a Shimadzu LC-6AD pump equipped with a Shimadzu SPD-10AV UV-VIS absorption detector set at 320 nm was used, and a Capcell Pak C18 column (3 mm inner diameter × 150 mm length; Shiseido Co., Ltd, Tokyo, Japan) was used as the analytical column. A mixture of acetonitrile and 10 mM CH_3COOH-CH_3COONa buffer of pH 7 (2:3, v/v) was used as the mobile phase and eluted at a flow rate of 0.5 mL/min. Each sample was injected onto the HPLC column with a volume of 20 µL. The concentration of CZ was determined by the absolute calibration curve method.

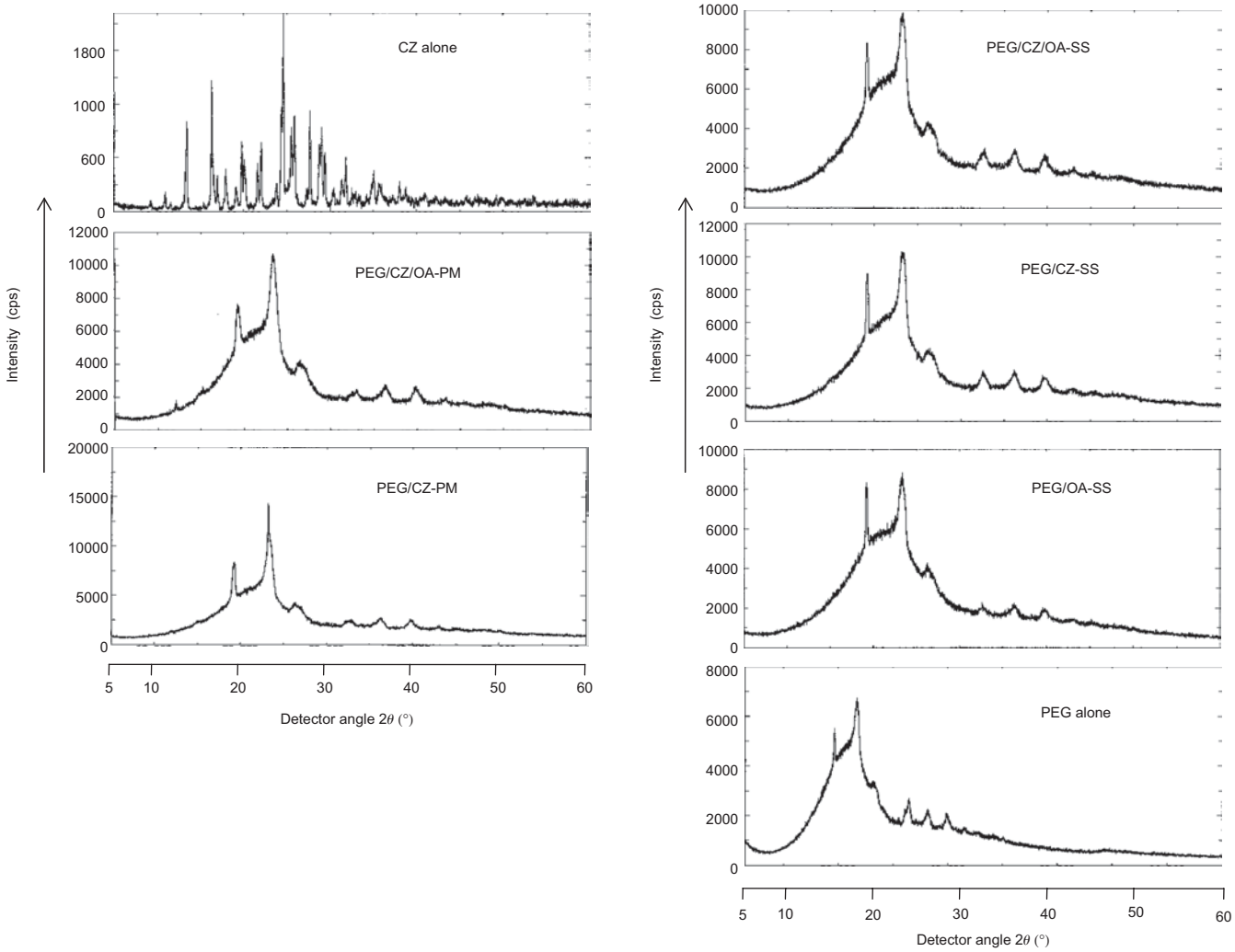

Figure 1. X-ray diffraction patterns of various formulations.

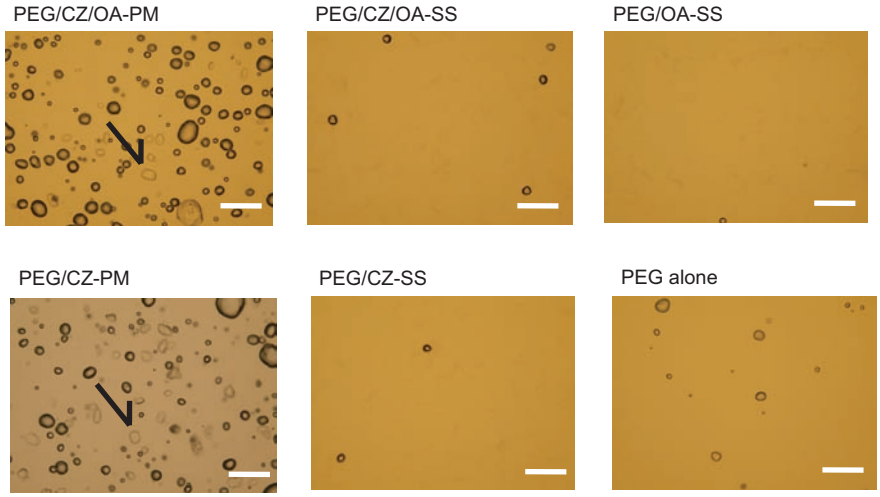

Figure 2. Photomicrographs of semi-solid formulations. The arrows show clonazepam (CZ) crystals. Dark ellipsoids in each photo are air bubbles. The length of white bar is 50 μm. PEG/CZ/OA-PM, physical mixture of polyethylene glycol 1500 (PEG), CZ, and oleic acid (OA); PEG/CZ-PM, physical mixture of PEG and CZ; PEG/CZ-SS; semi-solid dosage form of PEG and CZ; PEG/OA-SS, semi-solid dosage form of PEG and OA; PEG/CZ/OA-SS, semi-solid dosage form of PEG, CZ and OA.

Table 3. Detection of CZ recrystallization in the physical stability tests in a usual air condition (70% relative humidity, 20°C) for PG/CZ with or without OA and PEG/CZ with or without OA.

	Time after prepared				
Formulation	5 min	16 h	2 days	8 days	16 days
PG/CZ-D	–	+	+	+	+
PG/CZ/OA-D	–	+	+	+	+
PEG/CZ-SS	–	–	–	–	+
PEG/CZ/OA-SS	–	–	–	–	+

–:No crystal was detected in the dosage form microscopically (200×).
+:Crystals were detected in the dosage form microscopically (200×).
PG/CZ-D, droplet containing PG and CZ; PG/CZ/OA-D, droplet containing PG, CZ, and OA; PEG/CZ-SS, semi-solid dosage form of PEG and CZ; PEG/CZ/OA-SS, semi-solid dosage form of PEG, CZ and OA.
CZ, clonazepam; OA, oleic acid; PEG, polyethylene glycol 1500; PG, propylene glycol.

Results and discussion

Physical state and stability of semi-solid dosage forms

Drug powder, physical mixtures, semi-solid dosage forms, and their base were investigated by X-ray diffraction and microscopic observation. Only the drug powder showed diffraction peaks clearly (Figure 1). Other formulations showed almost the same diffraction patterns, and diffraction derived from CZ powder was not detected even with both the physical mixtures, PEG/CZ-PM and PEG/CZ/OA-PM. Diffraction peaks were found to be derived from the matrix base PEG, which was probably because a major component, PEG, governs diffraction. On the other hand, CZ crystals were observed well microscopically (×100 or ×200) in the physical mixtures (Figure 2). No CZ crystals were observed for PEG/CZ-SS, PEG/CZ/OA-SS, PEG/OA-SS and PEG alone. Thus, it was found out that the physical instability, that is, recrystallization from the matrix base, could be checked better by microscopic observation.

The recrystallization of each formulation is summarized in Table 3. Both PG/CZ-D and PG/DZ/OA-D exhibited solution state 5 min after cooling to room temperature; however, 16 h after cooling, small crystals were found in both formulations, consistent with previously reported results[11]. On the other hand, for PEG/CZ-SS and PEG/CZ/OA-SS, no crystals were observed in the matrix for at least 8 days after cooling to room temperature. The precipitation of crystals was observed on day 16 after cooling; therefore, the semi-solid dosage forms, PEG/CZ-SS and PEG/CZ/OA-SS, were physically stable for at least 8 days in air at room temperature.

Absorption after administration to oral mucosa

The absorption experiment from oral mucosa was performed as reported previously[11] except that a semi-solid dosage form was administered to the oral cavity between the lower gum and bottom lip. Semi-solid dosage forms gave the plasma concentration profiles as shown in Figure 3. Both PEG/CZ-SS and PEG/CZ/OA-SS showed the plasma levels at more than 20 ng/mL at 30 min or later. PEG/CZ-SS tended to show faster absorption than PEG/CZ/OA-SS, though their plasma levels were not significantly different at each time point ($P > 0.05$). For PEG/CZ-SS, the plasma level was highest and 32.0 ng/mL at 30 min, and decreased to 25.7 ng/mL at 60 min. On the other hand, after administration of PEG/CZ/OA-SS, the plasma concentration increased gradually, that is, it was 20.1 and 36.6 ng/mL at 30 and 60 min, respectively. The variation of the plasma concentration at each time point was less in PEG/CZ/OA-SS as compared with PEG/CZ-SS. The therapeutic plasma concentration threshold for anticonvulsant effects in humans is 10–20 ng/mL, according to the literature[7,9,15]. In addition, the effective plasma level was reported to be 20–30 ng/mL for rats[16]; therefore, both formulations were suggested to be possibly useful for the

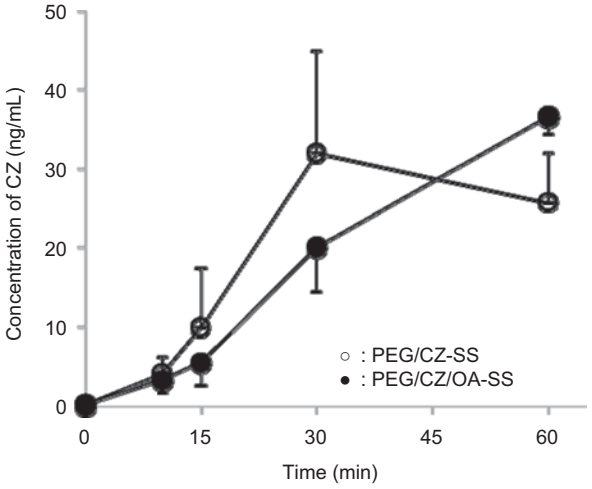

Figure 3. Plasma concentration of clonazepam (CZ) after buccal administration of semi-solid dosage form of PEG and CZ (PEG/CZ-SS) and semi-solid dosage form of PEG, CZ and OA (PEG/CZ/OA-SS). The results are expressed as the mean ± SE ($n=3$). OA, oleic acid; PEG, polyethylene glycol 1500.

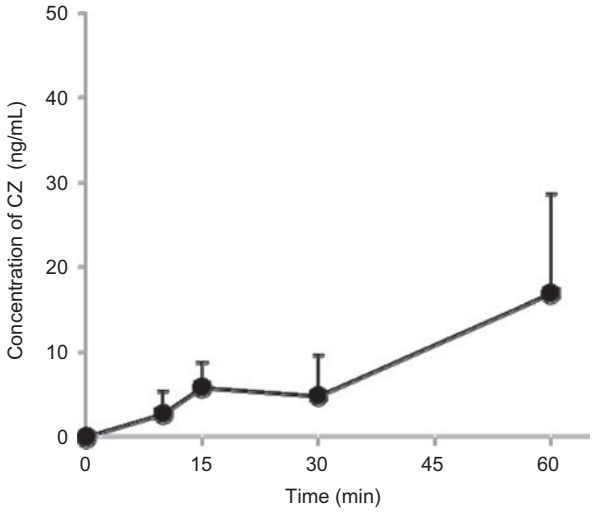

Figure 4. Plasma concentration of clonazepam (CZ) after sublingual administration of semi-solid dosage form of PEG, CZ and OA (PEG/CZ/OA-SS). OA, oleic acid; PEG, polyethylene glycol 1500. The results are expressed as the mean ± SE ($n=3$).

Table 4. Pharmacokinetic parameters for 0–60 min in oral mucosal absorption studies at 4 mg CZ/kg in rats.

Preparation	Administration site	C_{max} (0–60 min)	T_{max} (0–60 min)	*AUC* (0–60 min)	*MRT* (0–60 min)
PEG/CZ-SS	Buccal	33.7 ± 11.4	40.0 ± 10.0	1233.4 ± 432.7	39.7 ± 3.0
PEG/CZ/OA-SS	Buccal	36.6 ± 2.2	60.0 ± 0.0	1078.6 ± 197.6	45.2 ± 3.2
PEG/CZ/OA-SS	Sublingual	21.2 ± 9.3	35.0 ± 13.2	440.7 ± 173.2	36.4 ± 13.0

The results are expressed as the mean ± SE ($n=3$).
AUC, area under the plasma concentration time curve; MRT, mean residence time; PEG/CZ-SS, semi-solid dosage form of PEG and CZ; PEG/CZ/OA-SS, semi-solid dosage form of PEG, CZ and OA.

treatment of epileptic convulsions. As both semi-solid dosage forms melt quickly while absorbing fluid on the mucosal surface and in the oral cavity, CZ was considered to be absorbed quickly from the oral mucosa. Also, these results indicated that OA, which is often utilized as an enhancer for mucosal absorption of lipophilic or non-polar drugs[12–14], did not enhance the initial absorption rate. However, OA might contribute to the prolonged absorption because the plasma level increased gradually with PEG/CZ/OA-SS. In the present formulations, PEG was considered to function more importantly for the enhancement of the initial oral mucosal absorption.

In addition, after PEG/CZ/OA-SS was administered sublingually, the plasma concentration of CZ was monitored for 1 h. The plasma concentration was less than 10 ng/mL during the initial 30 min, and was nearly 20 ng/mL at 1 h (Figure 4). Furthermore, the plasma concentration varied widely; therefore, the sublingual administration of PEG/CZ/OA-SS was considered not to be appropriate for the absorption of CZ. As the mucosal features and fluid states are not uniform throughout the oral mucosa, this might influence the absorption rate. Since there is more fluid at a sublingual site than a buccal site, CZ is considered to precipitate more rapidly and spread faster at a sublingual site. Although the oral liquid was small in volume and scarcely observed to move into throat, a small portion of the drug might flow into the throat, especially in the sublingual administration. These could make the concentration gradients of CZ smaller in sublingual administration, leading to less absorption of CZ from a sublingual site, and might cause the greater deviation of the plasma concentration in the sublingual administration.

The pharmacokinetic parameters were obtained as shown in Table 4, in which maximum plasma concentration (C_{max}), time to show C_{max} (T_{max}), area under the plasma concentration time curve (*AUC*) and mean residence time (*MRT*) were calculated for 0–60 min, and *AUC* (0–60 min) and *MRT* (0–60 min) were calculated according to the trapezoidal method using a program MULTI[17]. An AUC (0–60 min) value was more than twice with buccal administration than sublingual administration. However, for each parameter, no significant difference was found among PEG/CZ-SS (buccal), PEG/CZ/OA-SS (buccal) and PEG/CZ/OA-SS (sublingual) ($P>0.05$).

Conclusion

The semi-solid dosage form of CZ could be prepared simply using PEG as a matrix base. Irrespective of addition of OA, PEG/CZ semi-solid dosage forms exhibited improved physical stability and effective plasma levels for anti-convulsion within 30 min after buccal administration. On the other hand, sublingual administration did not appear to be adequate for quick and stable

absorption. The present study can propose that buccal administration of semi-solid dosages, PEG/CZ and PEG/CZ/OA, should be possibly useful for the treatment of epileptic convulsions.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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