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Clonazepam Oral Droplets for the Treatment of Acute Epileptic Seizures

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Oral droplet formulations of clonazepam (CZ) were developed to examine their potentials as an alternative to i.v. administration for the treatment of acute epileptic seizures. Propylene glycol containing 2.5% (wt/wt) CZ with or without 5.0% (wt/wt) oleic acid (OA) was prepared as a solution by heating at 90°C and subsequently lowering the temperature to 30°C. The droplet (20 μL) was administered to the oral cavity between the lower gum and bottom lip before CZ precipitation started. With a droplet of propylene glycol loaded with 2.5% (wt/wt) CZ and 5.0% (wt/wt) OA, the plasma concentration reached 20 ng/mL (minimal effective concentration) within 10 min and was maintained between 20 and 60 ng/mL, less than a toxic level, for a period of 60 min. For a droplet of propylene glycol loaded only with CZ at 2.5% (wt/wt), it took more than 15 min for the plasma concentration to reach 20 ng/mL. It is suggested that a droplet of CZ/OA/propylene glycol (2.5:5.0:92.5, wt/wt) might be useful as an alternative to i.v. injection of CZ for the treatment of acute epileptic seizures.

Keywords clonazepam; oral droplet; plasma concentration; propylene glycol; oleic acid

INTRODUCTION

Benzodiazepines are generally used as sedating, antianxiety, or anticonvulsant agents. In particular, diazepam (DZ), lorazepam (LZ), and clonazepam (CZ) are widely used for the management of acute epileptic seizures or status epilepticus (Ashton, 1994; Rey, Treluyer, & Pons, 1999; Treiman, 1989). DZ and LZ are most frequently used for the treatment of these neurological disorders (Leppik et al., 2000; Li, Gorukanti, Choi, & Kim, 2000). Recently, the pharmacologic response to these drugs was reported to be directly associated with their plasma concentrations, indicating that an effective plasma concentration is required to control seizures (Brunton, Lazo, & Parker, 2005; Yasuda, 2004). Therefore, an emergent situation necessitates rapid drug absorption. The duration of convulsions before treatment initiation was critically correlated with the effectiveness; that is, in the treatment of children with rectally administered DZ, treatment within 15 min of onset was almost completely effective, but delayed treatment proved to be much less effective (Knudsen, 1979). Because i.v. administration can quickly deliver a precise amount of drug into the systemic circulation, it is considered to be the best way to achieve a rapid response. However, as i.v. administration requires a sterile condition, injection technique, etc., it is not convenient in emergent situations. Moreover, bolus i.v. administration may cause an excessive plasma concentration over the safety margin of the plasma level, resulting in toxic side effects such as psychomotor impairment, respiratory depression, and coma (Brunton, Pehourcq, & Jarry, 2005; Yasuda, 2004); therefore, other administration routes have recently been examined in an attempt to obtain an alternative to i.v. injection. Intranasal and rectal administrations have drawn attention as good candidates because they allow rapid absorption of drugs (Klosterskov Jensen, Abild, & Nøhr Poulsen, 1983; Li, Gorukanti, Choi, & Kim, 2000; Rylance, Poulton, Cherry, & Cullen, 1986; Scholss-Hendriks et al., 1995).

I.v. administration of CZ causes an effective pharmacologic response within 10 min, and the maximal plasma level (C_{max}) was reached within 2 min (Klosterskov Jensen, Abild, & Nøhr Poulsen, 1983; Li, Gorukanti, Choi, & Kim, 2000). Following intranasal administration, C_{max} is reached a little later, that is, at 10–20 min, though it was reported to be reached around 2 min in one case (Li, Gorukanti, Choi, & Kim, 2000; Scholss-Hendriks et al., 1995). Following rectal administration, it takes longer to reach C_{max}, around 10–30 min (Li, Gorukanti, Choi, & Kim, 2000). However, the C_{max} and the time to C_{max} (T_{max}) can be controlled by changes in formulation (Klosterskov Jensen, Abild, & Nøhr Poulsen, 1983; Li, Gorukanti, Choi, & Kim, 2000; Vyas, Babbar, Sharma, Singh, & Misra, 2006). Rapid achievement of therapeutic plasma levels is essential for effective treatment of acute epileptic seizures. Intranasal and rectal administrations rapidly achieve a high plasma level and can be an alternative route to i.v. injection. However, these administration routes may not be suitable because their administration manner is not necessarily easy in an emergency. Administration via oral mucosa is suggested as another possible alternative,

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though generally, absorption is not as rapid as that in intranasal or rectal administration. Previously, we developed a buccal patch containing DZ as a novel dosage form for the rapid absorption of DZ and achieved a plasma level of more than 200 ng/mL within 10 min after administration (Onishi, Sakata, Masuda, & Machida, 2005). However, as the effective plasma concentration of DZ is fairly high, the patch size required to achieve therapeutic plasma levels would have to be fairly large, which might limit its use. Also, as the duration of action is short (less than 2 h) for DZ, seizures may recur with the decline of the plasma level. Thus, CZ has become the focus of this study because its effective concentration is fairly low (20–70 ng/mL), much less than that of DZ, and the action duration of CZ is much longer (almost 24 h) (Brunton, Lazo, & Parker, 2005; Rey, Treluyer, & Pons, 1999; Yasuda, 2004). Droplets of CZ for administration to the oral mucosa between the lower gum and bottom lip were developed as a convenient formulation for the treatment of acute epileptic seizures and were evaluated for their usefulness based on their pharmacokinetics.

MATERIALS AND METHODS

Chemicals

Propylene glycol and CZ were purchased from Wako Pure Chemical Industries, Ltd. Oleic acid (OA) was supplied by NOF Corporation (Tokyo, Japan). Propylene glycol was used as a solvent, and OA was used as an additive for absorption enhancement (Onishi, Sakata, Masuda, & Machida, 2005; Tsutsumi, Obata, Takayama, Loftsson, & Nagai, 1998). All other chemicals used were of reagent grade.

Animals

Male Wistar rats weighing 250–260 g were purchased from Tokyo Laboratory Animal Science Co. Ltd. and soon used for experiments. The animals were kept on the breeding diet MF (Oriental Yeast Co., Ltd., Tokyo, Japan) with water ad libitum in a room where the temperature and relative humidity were maintained at 23 ± 1°C and 60 ± 5%, respectively, and the light–dark cycle was 12 h. The experimental protocol was approved by the Committee on Animal Research of Hoshi University (Tokyo, Japan). Animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals of Hoshi University.

Preparation of Oral Droplets

The droplet formulations of CZ, named oral droplets, are shown in Table 1. Propylene glycol containing CZ with no additives, named D-CZ-NO, was prepared as follows: CZ (5 mg) was mixed with propylene glycol (195 mg) and dissolved by heating in a water bath set at 90°C. Then, the temperature was decreased to 30°C in 5 min, and 30°C was maintained until the following experiments. The solution (20 μL) was taken up into an Eppendorf pipette and administered to an animal in the oral cavity between the lower gum and bottom lip. Propylene glycol containing CZ with OA, called D-CZ-OA, was prepared as follows. CZ (5 mg) was mixed with propylene glycol (185 mg), heated at 90°C until complete dissolution, and cooled to 30°C in 5 min. Then, OA (10 mg) was mixed with the solution and heated at 90°C for approximately 30 s. The mixture was then cooled in the same way as above. D-CZ-OA (20 μL) was administered in the same manner as in D-CZ-NO.

TABLE 1
Formulations of Oral Droplets

Constituent	D-CZ-NO (%, wt/wt)	D-CZ-OA (%, wt/wt)
Clonazepam	2.5	2.5
Propylene glycol	97.5	92.5
Oleic acid	0.0	5.0

In Vitro Physical Stability

After the temperature of the droplet formulation was decreased from 90 to 30°C, CZ was found to have slightly precipitated at the bottom of a test tube within the initial 60 min. The precipitate was only found in D-CA-OA test tubes. Therefore, precipitation of CZ from D-CZ-OA was monitored for 1 h just after the temperature reached 30 from 90°C. At appropriate time points, D-CZ-OA was centrifuged at 30°C at 1,500 g for 5 min, and an aliquot sample of the supernatant was taken. The sample was diluted with methanol, and the level of CZ was determined by high-performance liquid chromatography (HPLC).

In Vivo Absorption Studies

Rats were anesthetized by i.p. injection of pentobarbital solution in saline at 50 mg/kg (2 mL/kg). The administration was conducted within 30 min after the temperature of the droplet formulation decreased to 30 from 90°C. The droplet (20 μL) was administered to the oral cavity between the lower gum and bottom lip with an Eppendorf pipette. Blood samples (0.3 mL) were withdrawn immediately before and at 10, 15, 30, and 60 min after the administration. After the plasma was separated by centrifugation of the blood sample at 1,500 g for 10 min, 100 μL of the plasma was mixed with 0.2 mL of 0.1 M NaOH aqueous solution. Then, 4 mL of the hexane-ethyl acetate (9:1, vol/vol) mixture was added, shaken vigorously, and centrifuged at 1,500 g for 10 min. The organic phase (3.6 mL) was taken, and the solvent was evaporated completely under nitrogen gas. The residue was dissolved in 40 μL of the HPLC mobile phase, and 20 μL of the resultant solution was injected onto the HPLC column.

The recovery of CZ from plasma was determined as follows. Extraction from the plasma, to which the known amount of CZ

had been added, and subsequent treatment were conducted in the same way as for the tested plasma sample. The recovery ratio was calculated by comparison of the recovered amount with the ideal amount. The recovery ratio was almost 80%, independent of the concentration, and was used to correct the data.

For statistical analysis, a comparison was made using the unpaired *t*-test, and significant difference was set as $p < .05$.

HPLC Assay

The amount of CZ in the sample was determined by HPLC at room temperature by referring to the method of Bares, Pehourcq, and Jarry (2004). A Shimadzu LC-6AD pump equipped with a Shimadzu SPD-10AV UV-VIS absorption detector set at 320 nm was used. A Capcell Pak C18 column (3 mm in inner diameter×100 mm in length; Shiseido Co., Ltd., Tokyo, Japan) was used. A mixture of acetonitrile and 0.01 M sodium acetate buffer of pH 7 (2:3, vol/vol) was used as the mobile phase and eluted at a flow rate of 0.5 mL/min. The sample (20 µL) was injected onto the column. The concentration was calculated using the absolute calibration method. All data points were located within the linear region of the calibration curve.

RESULTS AND DISCUSSION

Preparation and Characteristics of Oral Droplets

As it was difficult to dissolve CZ in the present composition of each formulation at room temperature, CZ was dissolved at 90°C. At this temperature, CZ could be dissolved rapidly in both formulations (Table 1). When the solution is used as an oral droplet, better administration is possible when the temperature of the formulation is near the body or room temperature. Because of this reason, the solution temperature was decreased from 90 to 30°C in 5 min, which could be completed by hand. One formulation, D-CZ-NO, remained clear for at least 60 min after setting the temperature at 30°C. In other words, no precipitate was observed in that period. However, in regard to the other formulation, D-CZ-OA, a slight amount of the CZ solid was observed in the tube container at 60 min after being kept at 30°C. Therefore, the physical stability of D-CZ-OA as a supersaturated solution was examined at 30°C. As shown in Figure 1, immediately after the temperature was decreased to 30°C, the percentage of the CZ dissolved in the supernatant was almost 100% of the used CZ in each sample. This complete recovery of the used CZ also indicated that CZ is stable in the present treatment of heating at 90°C. At 30 and 60 min after the temperature was decreased to 30°C, 98 and 96% of the initial amount were observed in the supernatant, respectively. At 60 min, a slight amount of CZ solid was observed at the bottom of the tube. This might have been due to the fact that the amount of propylene glycol in the formulation was a little less in D-CZ-OA than in D-CZ-NO. OA appeared not to enhance the dissolution of CZ. Thus, in order to avoid the influence of CZ precipitation in the in vivo absorption studies, each formulation was administered within 30 min after its temperature was lowered from 90 to 30°C.

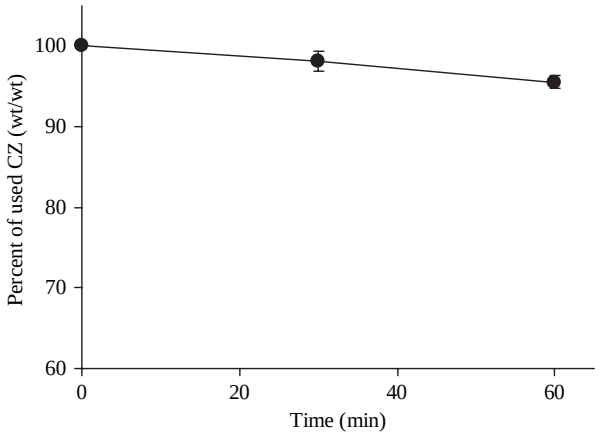

FIGURE 1. Percentage of clonazepam (CZ) dissolved in D-CZ-OA after reducing the temperature to 30°C. The results are expressed as the mean ± *SD* (*n* = 3).

In Vivo Absorption

Twenty microliters of the clear solution of the oral droplet formulation was withdrawn as described above and administered to the oral cavity between the lower gum and bottom lip. The plasma concentration–time profiles of CZ after administration are shown in Figure 2. During the initial 30 min, the mean plasma concentration with D-CZ-OA was approximately twice as high as that with D-CZ-NO. D-CZ-OA showed minimal effective concentration of CZ within 10 min. On the contrary, it took more than 15 min to achieve the concentration of 20 ng/mL with D-CZ-NO. Therefore, D-CZ-OA was

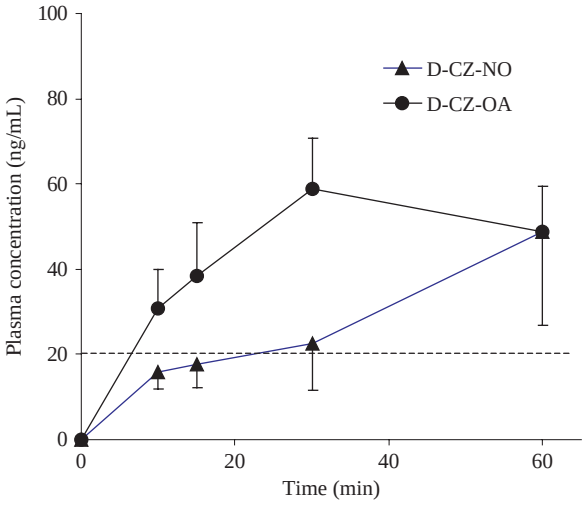

FIGURE 2. Plasma concentration of clonazepam (CZ) after administration of a droplet to the oral cavity of rats. The results are expressed as the mean ± *SE* (*n* = 3).

TABLE 2
Pharmacokinetic Parameters after Administration of Oral Droplets (20 μL) to the Oral Cavity of Rats

Patch	C_{max} (ng/mL)	T_{max} (min)	*AUC* (0–60 min) (mg min/mL)	*MRT* (0–60 min) (min)	*VRT* (0–60 min) (min^2)
P-DZ-NO	52.1 ± 21.0	50.0 ± 10.0	1.54 ± 0.45	40.8 ± 3.7	340.3 ± 30.8
P-DZ-OA	60.0 ± 10.9	40.0 ± 10.0	2.67 ± 0.54	34.6 ± 1.4	292.9 ± 14.3

Each value was caculated based on Figure 2.
The results are expressed as the mean ± SE (n = 3).

considered to be a better formulation to obtain a rapid response. At 60 min, both formulations showed almost the same concentration. CZ plasma levels of more than 100 ng/mL are considered to be in the toxic concentration range. Because D-CZ-OA showed the plasma levels of 20–60 ng/mL during 10–60 min, D-CZ-OA was considered adequate for rapid efficacy and suppression of toxic side effects. Furthermore, it was reported that a low dose of CZ was effective to reduce seizure frequency in children with epilepsy, in which a plasma level of 7–8 ng/mL is effective (Dahlin, Amark, & Nergardh, 2003). Considering this fact, D-CZ-NO might also be effective, because it exhibited a plasma concentration of more than 15 ng/mL during 10–60 min. These results suggest that oral droplets of CZ should be a suitable formulation to obtain a quick response, in particular, D-CZ-OA.

The pharmacokinetic parameters were calculated from the plasma concentration–time profiles. Maximal plasma concentration (C_{max}), time to reach C_{max} (T_{max}), area under the plasma concentration–time curve (*AUC*), mean residence time (*MRT*), and variance of residence time (*VRT*) were calculated for 0–60 min by the trapezoidal rule using the program called MULTI (Yamaoka, Tanigawara, Nakagawa, & Uno, 1981). The results are shown in Table 2. No significant difference was observed between the plasma concentrations of D-CZ-OA and D-CZ-NO at each time point ($p > .05$). Also, no significant difference was seen between both formulations for every pharmacokinetic parameter in Table 2 ($p > .05$). However, D-CZ-OA tended to exhibit a higher C_{max}, shorter T_{max}, and larger *AUC* (0–60 min), indicating more rapid and higher pharmacologic responses in D-CZ-OA than in D-CZ-NO.

Thus, the in vivo absorption study demonstrated present CZ formulations, in particular, D-CZ-OA, could achieve effective plasma concentration quickly, though, as stated above, the present formulations have a problem with physical stability. The precipitate appearing in the formulation can be dissolved easily by re-heating the formulation. Therefore, in the circumstances where the formulation can be heated any time, it will be available immediately when the seizure occurs. But, in the other situations, the availability of the formulation may be limitted due to the solubility problem. This problem may be solved by further formulation studies. This point will be a future subject for the practical use.

CONCLUSION

An oral droplet formulation, containing CZ and OA at 2.5 and 5.0% (wt/wt), respectively, was prepared using propylene glycol as a solvent. When the droplet (20 μL) was administered to the oral cavity between the lower gum and bottom lip in rats, the plasma concentration reached 20 ng/mL within 10 min and was maintained between 20 and 60 ng/mL for a period of 60 min. Therefore, this formulation (D-CZ-OA) was considered to be suitable for a quick response (effective plasma concentration range: 20–70 ng/mL) and non-toxic (toxic plasma concentration range: >100 ng/mL). D-CZ-OA is suggested to be a possible alternative to i.v. injection in the emergency of epileptic seizures. In addition, the formulation composed of only CZ and PG (2.5:97.5, wt/wt) may also be effective, because it showed a plasma level of more than 15 ng/mL at 10 min. Thus, it is suggested that the present formulations, in particular, a CZ/OA/(propylene glycol) mixture, should be useful for the treatment of epileptic seizures. The present formulations will have to be elucidated for their clinical usefulness in more detail by further examination of practical points such as amount and accuracy. Also, more detailed formulation studies to solve the problem with solubility of CZ may be necessary to enable practical use of the CZ/OA/(propylene glycol) mixture formulation in various situations.

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