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Novel Mucoadhesive Oral Patch Containing Diazepam

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ABSTRACT The oral patch of diazepam (DZ) was developed to achieve a rapid absorption of DZ for the emergency treatment of epileptic seizure or anxiety disorder. The patch was composed of the outer mucoadhesive Carbopol 934 region, central drug region, and Tegaderm backing film. DZ (3 mg) was dissolved in propylene glycol (PG) alone or PG containing oleic acid (OA) at 5.6% (w/w), and used as the drug region. The patches with and without OA were attached to the mucosa of cheek in rats. The patch with OA exhibited the plasma level of more than 200 ng/mL at 10 min after administration, then the plasma concentration decreased gradually. The patch without OA displayed a plasma level of less than 30 ng/mL during 40 min after administration. To the contrary, in the in vitro drug permeation using a cellulose membrane, the patch without OA showed a three times faster permeation rate than the patch with OA, suggesting that the direct action of OA to mucosa might be associated with absorption enhancement. It was demonstrated that the patch with OA showed a good adhesion to oral mucosa and worked efficiently for rapid absorption of DZ.

KEYWORDS Mucoadhesive oral patch, Diazepam, Oleic acid, Rapid absorption, Plasma level

INTRODUCTION

Diazepam (DZ) is widely used as a sedative, anti-anxiety medication, and anticonvulsant (Bechgaard et al., 1997; Gizurarson et al., 1999; Rey et al., 1999). Furthermore, DZ is known to be useful in emergency situations in which suppression of convulsions or anxiety disorders is required (Knudsen, 1979; Li et al., 2000). In such treatment in emergency, rapid absorption of DZ is required (Knudsen, 1979; Li et al., 2000). It has been reported that the therapeutic effect was correlated with the duration of convulsions before treatment; that is, early treatment of convulsions within 15 min of onset resulted in very high efficacy, but late treatment did not show a positive effect (Knudsen, 1979). I.V. administration is the most rapid way to complete suppression of neurological disorders. However, i.v. administration is not necessarily simple or easy because a sterile syringe, injection technique, etc. are required (Li et al., 2000) and because excessive concentration, leading to toxic side effects such as respiratory depression (Norris et al., 1999; Ogutu et al.,

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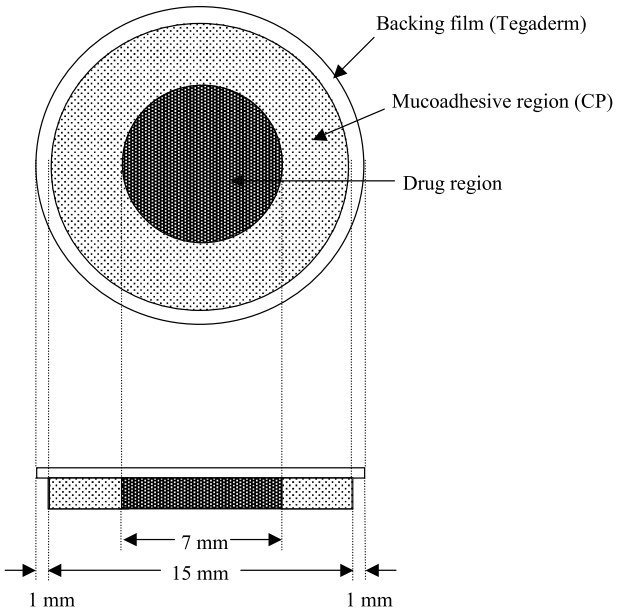

FIGURE 1 **Schematic Diagram of Oral Patch.**

2002), may be caused. Therefore, other administration routes have been examined in an attempt to achieve rapid absorption of DZ. Rectal administration has been reported as a useful method in emergent treatment of convulsions (Agurell et al., 1975; Knudsen, 1979; Mattila et al., 1981; Meberg et al., 1978; Moolenaar et al., 1980). It does not appear to be as strongly effective as i.v. administration, but considerably effective, and causes few toxic side effects. However, rectal insertion is not necessarily convenient in emergency situations. Recently, great attention has been paid to nasal administration because it can attain a high bioavailability in absorption rate and extent (Bechgaard et al., 1997; Gizurarson et al., 1999; Li et al., 2000). In nasal absorption, the volume of the solution administered is limited, and the vehicles are important for achieving rapid absorption of DZ (Li et al., 2000). Although nasal administration is good for rapid absorption, that way may not be necessarily convenient due to the manner of application. Thus, in this study, a novel oral patch allowing the administration of DZ to oral mucosa was developed to achieve rapid absorption up to an effective concentration. This dosage form can be applied simply, localized at the attached site, and easily removed. The mucoadhesive polymer Carbopol 934 (CP) (Chary et al., 1999; Park & Munday, 2002) was used as a mucoadhesive region of the dosage form, and propylene glycol (PG) was employed as a vehicle for its drug region because DZ could dissolve well in PG. Further, oleic acid (OA) was tested as an additive that has the potential to enhance the absorption via oral mucosa (Birudaraj et al., 2005; Manganaro & Wertz, 1996; Shojaei, 1998; Tsutsumi et al., 1998). The patches with and without OA were examined on absorption through oral mucosa after attachment to the mucosa of cheek in rats. Further, their drug release properties were investigated in vitro using a cellulose membrane.

MATERIALS AND METHODS

Chemicals

Diazepam (DZ), propylene glycol (PG), and oleic acid (OA) were purchased from Wako Pure Chemical Industries, Ltd. Carbopol 934 (CP) was obtained from Mitsuya Chemical Co. All other chemicals used were of reagent grade.

Animals

Male Wistar rats (350–400 g) were purchased from Tokyo Laboratory Animal Science Co., Ltd. (Japan), and soon used for experiments. The animals were kept on the breeding diet MF (Oriental Yeast, Japan) with water ad libitum at room temperature maintained at 23±1°C and a relative humidity of 60±5%. The experimental protocol was approved by the Committee on Animal Research of Hoshi University, Tokyo, Japan, and the animal experiments were performed in compliance with Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, Japan.

TABLE 1 **Formulations of Oral Patches With and Without Oleic Acid**

		Drug region		
Patch	Mucoadhesive region (CP) (mg/patch)	DZ (mg/patch)	PG (mg/patch)	Oleic acid (mg/patch)
P-DZ-NO	60	3	45	–
P-DZ-OA	60	3	42.5	2.5

Preparation of Oral Patches

The oral patch, the schematic illustration of which is described in Fig. 1, was prepared with the formulation as shown in Table 1. Namely, 60 mg of CP fine powder was compressed into a thin circle plate with a diameter of 15 mm, using a Shimadzu Hand Press HP (Shimadzu, Japan) at 40 kg/cm^2 for 1 min. Then, a hole of 7 mm was made in the central region of the plate using a cork borer. The thin plate with a hole in the middle was pressed on the adhesive face of a Tegaderm film (3M, USA). Then, DZ (3 mg) was dissolved in PG (45 mg) alone or in the mixture of PG (42.5 mg) and OA (2.5 mg), and the solution was put in the hole to which a small amount of cotton fiber (1 mg) was placed in advance. Finally, the Tegaderm film was cut along with the outside edge of the CP region, and used soon for in vivo and in vitro experiments.

In Vivo Absorption Studies

After fasting for 20 h, rats were anesthetized by i.p. injection of the saline solution of sodium pentobarbital at 50 mg/kg (2 mL/kg). The CP part of the patch was attached to the mucosa of cheek at the condition of one patch per rat. Blood sampling (each 350 μL) was performed immediately before administration and at 10, 20, 40, and 60 min after administration. The plasma was obtained by centrifugation of the blood at 3000 rpm for 10 min. The plasma sample was treated in reference to the method by Muchohi et al. (2001).

Five hundred microliters of 0.1 M borate buffer, pH 9.0, was mixed in 100 μL of the plasma. Then, 5 mL of the mixture of ethyl acetate and n-hexane (3:7, v/v) was added to the plasma-buffer mixture, shaken vigorously, and centrifuged at 2500 rpm for 5 min. The whole organic layer was taken, and the solvent was evaporated completely at room temperature under nitrogen gas. The residue was dissolved in 100 μL of the HPLC (high performance liquid chromatography) mobile phase, and assayed by HPLC on the DZ concentration. The recovery of DZ from the plasma was 80%, and the plasma concentration was corrected with this recovery ratio.

Pharmacokinetic Analysis

The area under the plasma concentration-time curve AUC(0–60 min), mean plasma residence time MRT(0–60 min), and variance of residence time VRT(0–60 min), expressed by the following equations, were calculated using the trapezoidal method.

$$AUC(0\text{–}60\ \text{min}) = \int_0^{60} C\text{p}\,\text{d}t \tag{1}$$

$$MRT(0\text{–}60\ \text{min}) = \int_0^{60} (C\text{p} \times t)\text{d}t / AUC(0\text{–}60\ \text{min}) \tag{2}$$

$$VRT(0\text{–}60\ \text{min}) = \int_0^{60} (C\text{p} \times (t - MRT(0\text{–}60\ \text{min}))^2)\text{d}t / AUC(0\text{–}60\ \text{min}) \tag{3}$$

where Cp meant the plasma concentration at time t (min). The calculation was performed using the program MULTI (Yamaoka et al., 1981).

In Vitro Permeation Studies

The in vitro release study was performed using Franz-type diffusion cells. The cellulose membrane (MW cut off=14,000) was set as a permeation membrane. Phosphate buffered saline, pH 7.4, (PBS) (27 mL) was put in the chamber for the solvent upper surface to reach the bottom of the membrane completely, and the medium was stirred fast and constantly using a magnetic stirrer. Then, the patch was attached to the center of the upper side of the membrane for the Tegaderm backing film to be located outside. At appropriate time points, aliquot samples (1.5 mL) were taken, and the same volume of fresh PBS was supplemented. Each sample was diluted adequately with PBS, and measured by HPLC on the concentration of DZ.

HPLC Assay

HPLC assay was performed at room temperature as follows: A Shimadzu LC-6A equipped with a UV-VIS absorption detector Shimadzu SPD-10AV set at 250 nm was used for analysis. A Shimadzu C-R7A plus Chromatopac was used as a recorder and data processor. The columns, ULTRASPHERE ODS 5 μ (4.6 mm in inner diameter × 150 mm in length; Beckman, USA) and Capcell Pak C18 (3 mm in inner diameter × 10 cm in length; Shiseido, Japan), were used

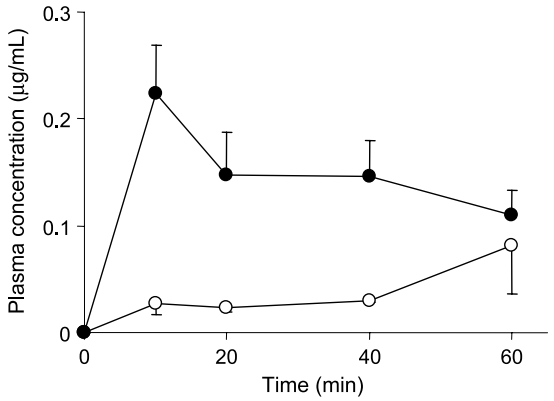

FIGURE 2 **Plasma Concentration-Time Profiles of DZ After Attaching Oral Patches to the Mucosa of Cheek in Rats. ○, P-DZ-NO; ●, P-DZ-OA. The Results are Expressed as the Mean ± S.E. (n = 3).**

for in vivo and in vitro analyses, respectively. The mixture of 25 mM phosphate buffer, pH 6, and methanol (45:55, v/v) was used as a mobile phase. The flow rates of the mobile phase were set at 1.2 mL/min and 0.3 mL/min for the ULTRASPHERE and Capcell Pak columns, respectively. Twenty μL of the sample was injected on those columns. The calculation of concentration was performed using the absolute calibration curve method. All the data points were located within the linear region of the calibration curve.

RESULTS AND DISCUSSION

Physical Properties of Patches

First, CP was compressed using a manual tableting machine to make a mucoadhesive region. The thin CP plate, which was obtained under the condition of the compression force of 40 kg and compression time of 1 min, was not fragile but a little bendy. After the CP plate stood overnight, it got bendier by adsorption of air moisture. The plate could be holed without breakdown using a cork borer. Further, this bendy property of the CP region was considered to allow the patch to stick to a curved surface. PG, which is known as an organic solvent of very low acute toxicity and often used as a food additive, was useful to dissolve DZ. One milligram of cotton fiber, attached to the hole (drug region), could retain PG up to approximately 50 mg. Therefore, 45 mg of PG or the PG/OA mixture was used as the medium for DZ, and 3 mg of DZ was dissolved in the medium by mild warming for a short time and put in the hole. DZ is considerably stable unless it is treated with strongly acidic or strongly basic conditions (Han et al., 1977; Kowaluk et al., 1983; Morris, 1978). Therefore, DZ was considered to be kept fairly stable in the patch because the media used were not strongly acidic or strongly basic.

In Vivo Absorption

After attachment of the patch to the mucosa of cheek in rats, the plasma concentration profiles of DZ were observed as shown in Fig. 2. The patch without OA (P-DZ-NO) did not exhibit a rapid increase in plasma concentration. The plasma level was kept at 24–30 ng/mL during 40 min after administration, and increased up to 83 ng/mL at 60 min after administration. These plasma levels given by P-DZ-NO were less than the pharmacologically effective levels observed in rabbits or human (Li et al., 2000; Ogutu et al., 2002). On the other hand, the patch with OA (P-DZ-OA) showed a high plasma concentration at 10 min after administration, which was 223 ng/mL. Then, the plasma level decreased gradually, and reached 110 ng/mL at 60 min after administration. The initial level, more than 200 ng/mL, was within or near pharmacologically effective levels observed in rabbits or human (Li et al., 2000; Ogutu et al., 2002). These suggest that OA should greatly enhance the absorption rate of DZ

TABLE 2 **Pharmacokinetic Parameters After Administration of Oral Patches to the Mucosa of Cheek in Rats**

Patch	C_{max} (ng/mL)	T_{max} (min)	*AUC* (0–60 min) (μg·min/mL)	*MRT* (0–60 min) (min)	*VRT* (0–60 min) (min^2)
P-DZ-NO	86.1 ± 42.1	43.3 ± 16.7	2.04 ± 0.35	38.70 ± 5.73	286.60 ± 32.11
P-DZ-OA	251.0 ± 18.4[a]	13.3 ± 3.3	8.47 ± 0.65[b]	29.46 ± 0.51	288.21 ± 46.03

The results are expressed as the mean ± S.E. (n = 3).
[a] $P < 0.05$.
[b] $P < 0.001$.

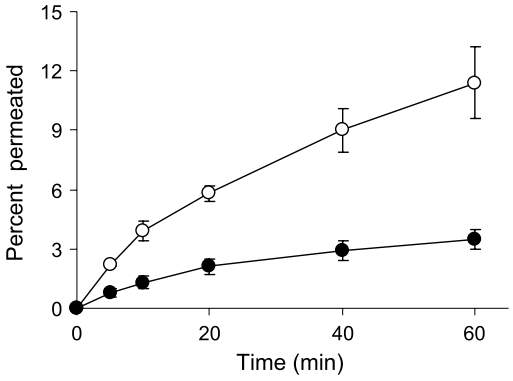

FIGURE 3 **In Vitro Permeation of DZ from Oral Patches Through the Cellulose Membrane. ○, P-DZ-NO; ●, P-DZ-OA. The Results are Expressed as the Mean ± S.D. (n = 3).**

from the mucosa of cheek, and that the plasma concentration might rapidly reach the pharmacologically available level by P-DZ-OA, though the problem with the difference in kinds of animals remains to be solved.

The patches stuck well to the mucosa of cheek while they were applied. They were removed from the mucosa of cheek after blood sampling at 60 min after administration. After removing the patch, no damage such as inflammation was observed at the site of administration.

Pharmacokinetic Analysis

The pharmacokinetic parameters are calculated as described in Table 2. When OA was added, T_{max} was reduced to the value of less than one-third, and C_{max} was raised approximately three times more. Significant difference was observed for the C_{max} values between P-DZ-NO and P-DZ-OA ($p<0.05$). *AUC*(0–60 min) was more than four times greater significantly in P-DZ-OA than in P-DZ-NO ($p<0.001$). *MRT*(0–60 min) and *VRT*(0–60 min) were not significantly different between them.

In Vitro Release

Soon after attachment to the cellulose membrane, the CP and drug regions became wet, and the patch stuck to the membrane fairly firmly. DZ permeated the membrane gradually for both the patches (Fig. 3). During the in vitro experiment, a Tegaderm film prevented the drug and solvent from leaking out of the upper surface. The percent permeated from P-DZ-NO reached 11.4% at 60 min after the attachment, which showed the amount of the DZ permeated after the attachment for 60 min was 342 μg. On the other hand, P-DZ-OA showed a drug permeation of 3.5% at 60 min after the attachment. This indicated that the permeation rate was reduced to approximately one-third by the addition of OA. DZ solution in PG, prepared under this experimental condition, kept the solution clear after the addition of OA, that is, OA did not appear to prevent the dissolution of DZ in solvent. Immediately after the patch was attached to the membrane, PBS permeated the drug region. Therefore, the physical condition of the drug region was investigated when PBS was added to the PG solution containing DZ with or without OA. When PBS was added gradually to the PG solution containing DZ and OA, the solution rapidly became cloudy. On the other hand, such change did not occur when the PG solution containing only DZ was treated by the same way. Therefore, P-DZ-OA was considered to be emulsified rapidly by mixing with PBS, which might become a barrier against the permeation through the membrane. The difference in the physical change caused by mixing with PBS was suggested to influence the in vitro permeation profile. Further, as the medium of the drug region diffused into the CP region during the membrane permeation, this appeared to suppress the permeation of DZ through the membrane. This point remains to be studied in the future.

Although the release studies show that the addition of OA lowered the permeation rate of DZ through the cellulose membrane, the in vivo absorption of DZ was enhanced obviously by addition of OA. Therefore, the direct action of OA to the mucosal membrane was considered to be associated with enhancement of absorption in vivo. Recently, mucosa of cheek excised from hamsters, pig, etc. has been reported to be applied to the in vitro permeation studies to investigate the detailed mechanism of the permeation (Artusi et al., 2003; Birudaraj et al., 2005; Shojaei, 1998; Tsutsumi et al., 1998; Veuillez et al., 2002). The present results only could give the comparison of release properties, and the use of excised mucosal membrane is expected to present the results different from those obtained here with the cellulose membrane because of the physicochemical and biological features of the mucosal membrane itself. In many reports, the PG is used as a component of the media of the dosage forms applied to skin and oral mucosa (Birudaraj et al.,

2005; Rosado et al., 2003; Shokri et al., 2001; Tsutsumi et al., 1998), and can increase the solubility of the drugs (Birudaraj et al., 2005; Tsutsumi et al., 1998). Further, absorption enhancers are often added to the media because the mucosa of cheek is not necessarily permeable enough to obtain high bioavailability (Shojaei, 1998). OA appears to be useful for enhancement of absorption of lipophilic drugs, and combination of PG and OA seems to give the better transport through the mucosal membrane (Birudaraj et al., 2005). Elaborate dosage forms, produced with mucoadhesive polymers, absorption enhancers, etc., have been developed as the drug delivery systems applied to the oral mucosa (Shojaei, 1998). The present patch is proposed as a useful mucoadhesive oral dosage form to transport DZ up to the pharmacologically effective level in the systemic circulation.

CONCLUSION

In the patch prepared in this study, the outer mucoadhesive CP region exhibited good adhesion to the mucosa of cheek, the vehicle PG allowed DZ to dissolve well, and the backing Tegaderm film operated efficiently to prevent the drug and solvent from leaking out of the outer surface. The patch with the drug region composed of PG, DZ, and OA at 42.5, 3, and 2.5 mg, respectively, showed a rapid absorption, that is, the plasma level was more than 200 ng/mL at 10 min after the attachment of the mucosa of cheek in rats, which was within or near the effective concentration in rabbits or human. On the other hand, the patch with no OA exhibited much slower and lower absorption. The in vitro permeation studies using a cellulose membrane suggested that the direct action of OA to the mucosal membrane should play an important role in enhancement of in vivo absorption. The present mucoadhesive oral patch having the PG/DZ/OA mixture as the drug region is proposed as a possibly useful dosage form for emergency treatment of epileptic seizure and anxiety disorder.

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