

Membrane-Controlled Transdermal Therapeutic System Containing Clonazepam and Anticonvulsant Activity after Its Application

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A trial transdermal dosage form designed to sustain a suitable plasma concentration of clonazepam (CZP) was produced using a porous membrane (Hipore 2100 or 4050) and applied to rabbits and rats for pharmacokinetic and pharmacodynamic evaluations. The release rate constants for the drug through the porous membranes were significantly smaller than that without any membrane. The transdermal system (Hipore 4050 system, ointment 0.25 g, 2.25 cm²) provided a well sustained plasma concentration of CZP and the therapeutic plasma concentration range was maintained for about 26 h. When the Hipore 4050 system with an increased amount of ointment and enlarged absorption area (0.5 g, 4.0 cm²) was applied, the therapeutic range was sustained for about 40 h, and slightly higher plasma levels over the whole application period and much higher bioavailability (37%) were obtained compared with those after the 2.25 cm²-Hipore 4050 system. The transdermal system exerted an excellent anticonvulsant activity in rats, with the best (3+ or 4+) protective score. The plasma concentrations of CZP when the activity was estimated were in the therapeutic range. Thus, the transdermal system has the potential to be an efficient drug delivery system.

Keywords clonazepam; controlled release; microporous membrane; drug delivery system; transdermal therapeutic system; antiepileptic activity; pharmacokinetic evaluation; pharmacodynamic evaluation

To maintain a constant level of a drug in the blood or target tissue is the ideal goal of a controlled drug delivery. The result of obtaining a constant drug blood level from a sustained- or controlled-release system is to achieve promptly and maintain the desired effect. Additionally, reduction or elimination of fluctuations in the drug level allows better disease state management. Minimizing or eliminating the patient compliance problem is also an obvious advantage of sustained- or controlled-release therapy.

One of the approaches for transdermal drug dosage forms acting long term is the use of microporous membranes as rate-controlling barriers. A microporous membrane has been used to control the release from drug preparations effective for the treatment of angina pectoris¹⁾ and motion-induced nausea.²⁾

Clonazepam (CZP) has anticonvulsant properties in several animal species.³⁾ CZP has been used in a once to three times a day oral administration regimen in man, although it has a long half-life (18.7 to 39 h⁴⁾ and 19 to 60 h⁵⁾ in man). An attempt to extend the duration of action of CZP would be beneficial for patients.

In this study, a trial transdermal dosage form designed to sustain the plasma concentration of CZP within the therapeutic concentration range was produced using a microporous membrane as the rate-controlling barrier and applied to rabbits and rats for pharmacokinetic and pharmacodynamic evaluations.

Experimental

Materials CZP and Azone® were supplied by Sumitomo Chemical Co., Ltd. and Nelson Research and Development Co., respectively. Diazepam and sodium valproate were generous gifts of Takeda Pharmaceutical Co., Ltd. and Kyowa Fermentation Industry Co., Ltd., respectively. The porous membranes used were Hipore 2100 (mean pore size: 0.23 μm, membrane thickness: 100 μm) and 4050 (mean pore size: 0.30 μm, thickness: 50 μm) made of polyolefin, which were produced by Asahi Organic Chemicals. An adhesive tape (No. 750, 50 mm × 25 m) was purchased from Nitto Denko Co. The transdermal therapeutic system depicted in Fig. 1 was used. The absorption area of the system was 1.5 × 1.5 cm (2% CZP ointment, 0.25 g) or 2.0 × 2.0 cm (2% CZP ointment, 0.5 g). In the

experiment using rats, the system with the absorption area of 0.5 × 0.5 cm (2% CZP ointment, 0.12 g) was prepared and utilized.

Animals Male Japanese white rabbits, weighing 2.5 to 3.5 kg, were used throughout this experiment. Male Wistar rats, weighing 150 to 170 g for estimating the antiepileptic effect and weighing 230 to 250 g for measuring the plasma drug concentration, were also used.

Preparation of Ointment CZP ointment (CZP 2.0%, carbital 39.0%, propylene glycol 10.0%, Hiviswako 1.0%, Azone 4.0%, sorbitan monooleate 4.0%, diisopropanolamine 1.1%, diisopropyl adipate 2.0%, (w/w), Rp. 3)⁶⁾ was prepared by the same method as described in the previous paper.⁶⁾

Percutaneous (p.c.) Administration The transdermal therapeutic system was applied to the back area of a rabbit, from which the hair had been removed on the day before the experiment, for 36 or 54 h. The application of ointment (0.12 g, 0.25 cm²) to rat abdominal skin was done for 24 h under occlusion by the same method as described previously.⁷⁾

Determination of CZP CZP in plasma samples was determined by high-performance liquid chromatography as described in the previous paper.⁶⁾ The limit of detection of CZP in plasma was approximately 7 ng/ml.

Drug Release through Filter Paper and Microporous Membrane from Ointment The drug release from ointment was estimated by using a Franz diffusion cell with a 3.5 cm i.d. O-ring flange.⁸⁾ A Hipore membrane or a filter paper (Toyo Filter Paper, No. 5C) spread with 2.0 g of ointment was mounted on a diffusion assembly and the drug that penetrated into the receptor fluid (0.9% NaCl-10 mM phosphate buffer, pH 7.4) at 37°C was determined for 10 h by the method described previously.⁶⁾

Measurement of Anticonvulsant Activity Male Wistar rats were divided at random into 5 groups, each consisting of 5 rats. One group was assigned as the control group and received sodium valproate (100 mg/kg, valproic acid equivalent) solution orally. Valproate was chosen as a broad-spectrum anticonvulsant. The second group was treated with the placebo ointment (0.12 g, 0.25 cm²). The third group was given 2% CZP ointment (0.12 g, 0.25 cm²) and the fourth and fifth groups were given the transdermal system. Pentetrazole (95 mg/kg) dissolved in saline was administered

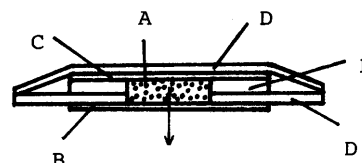


Fig. 1. Sectional View of Transdermal Therapeutic System

A, ointment (2% CZP); B, porous membrane (Hipore); C, aluminum foil; D, adhesive tape; E, silicon rubber membrane.

subcutaneously 1 h after dosing for the control group, 4 h post-application for the second and third groups and 10 h post-application for the fourth and fifth groups, and then the strength and occurrence of convulsion were observed for 1.5 h. The anticonvulsant activity in each group was measured at a time giving a relatively high plasma drug concentration. The convulsion was classified into three categories, slight, moderate and frank, and the anticonvulsant effect was evaluated according to the protective score (0 to 4+).⁹⁾

Pharmacokinetic and Statistical Analyses The pharmacokinetic parameters after intravenous (i.v.) administration (0.2 mg/kg) were obtained from our previous study.⁶⁾ The area under the plasma drug concentration-time curve ($AUC_{0-\infty}$) was calculated by means of the following equation¹⁰⁾:

$$AUC = \int_0^t C_p dt + \int_t^\infty C_t \cdot e^{-k_e t} dt$$

where C_t and k_e are the plasma concentration at the last sampling point and the terminal elimination rate constant calculated using the last 3–4 data points, respectively.

In the *in vitro* release experiment, we assumed that the release process was first-order. The release rate constant was calculated by applying the following equation:

$$C_t = C'_i(1 - e^{-k_r t})$$

where C'_i and C_t are drug concentrations in the receptor fluid at time t and at infinite time, respectively, and k_r is the release rate constant.

All data were analyzed with the non-linear iterative least-squares regression analysis program, MULTI.¹¹⁾ The means of all data are presented with their standard deviation (mean \pm S.D.). Statistical analysis was performed by using the non-paired Student's t test and a p value of 0.05 or less was considered to be significant.

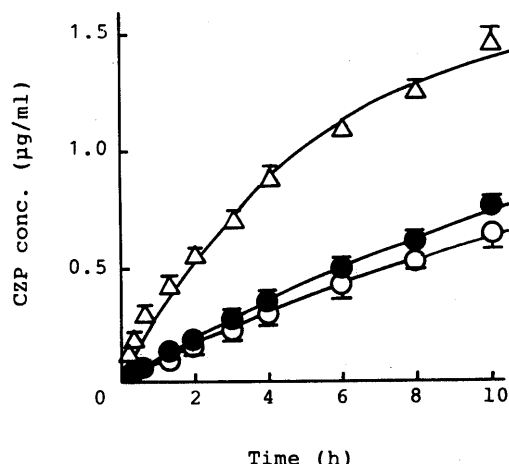


Fig. 2. Permeation of CZP through Porous Membrane from Ointment

Each point represents the mean \pm S.D. of 3 experiments. Δ , filter paper; \bullet , Hipore 4050; \circ , Hipore 2100.

Results

Drug Release through Microporous Membrane The drug release through Hipore membranes was examined by using a Franz diffusion cell. The *in vitro* release profiles of CZP through the membranes or a filter paper are shown in Fig. 2. The values of release rate constant, k_r , and C'_i are shown in Table I. The rate constants for drug through the porous membranes were significantly smaller than that without the membrane, indicating that the membranes do act as a controlled-release barrier.

Sustaining Plasma Concentration of CZP by Use of a Microporous Membrane In this study, an attempt has been made to sustain the plasma concentration of CZP by using a microporous membrane as a rate-controlling barrier for the drug delivery system, based on the result obtained in the previous study.⁶⁾ The time course of plasma concentration

TABLE I. Release Parameters of CZP through Porous Membranes

Parameter	Rp. 3	Hipore 2100	Hipore 4050
C'_i ($\mu\text{g/ml}$)	16.33 ± 0.72	14.99 ± 5.08	14.91 ± 2.15
k_r (h^{-1})	0.393 ± 0.053	$0.124 \pm 0.053^a)$	$0.153 \pm 0.034^a)$

a) $p < 0.02$ compared with Rp. 3.

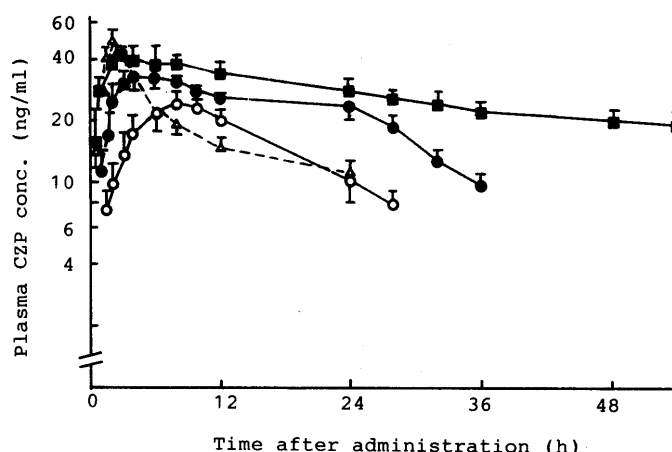


Fig. 3. Plasma Concentration of CZP after Percutaneous Application of Membrane-Controlled System

Each point represents the mean \pm S.D. of 4 experiments. The application of systems was for 36 h (2% CZP ointment, 0.25 g/2.25 cm²) and 56 h (2% CZP ointment, 0.5 g/4.0 cm²). \bullet , Hipore 4050 system (0.25 g/2.25 cm²); \blacksquare , Hipore 4050 system (0.5 g/4.0 cm²); \circ , Hipore 2100 system; Δ , 2% CZP ointment.

TABLE II. Pharmacokinetic Parameters of CZP after Percutaneous Application of Membrane-Controlled System

Parameter	Rp. 3	Membrane-controlled system		
		Hipore 2100	Hipore 4050 (0.25 g/2.25 cm ²)	Hipore 4050 (0.5 g/4 cm ²)
C_{\max} (ng/ml) ^{a)}	49.6 ± 3.7	$24.1 \pm 5.1^f)$	$33.8 \pm 2.1^{g,h)}$	40.9 ± 5.6
T_{\max} (h) ^{b)}	2.0	7.5	4.8	3.0
$t_{1/2}$ (h) ^{c)}	21.1 ± 7.3	$11.3 \pm 0.6^f)$	$8.8 \pm 2.7^f)$	$97.6 \pm 11.1^i)$
AUC (ng \cdot h/ml)	801.3 ± 177.4	$541.4 \pm 104.7^f)$	$936.1 \pm 46.8^h)$	$3024.0 \pm 615.6^i)$
Bioavailability ^{d)}	21.9 ± 4.6	$14.9 \pm 2.9^f)$	$25.8 \pm 1.3^h)$	$37.2 \pm 4.0^i)$
Duration time (h) ^{e)}	6.9 ± 0.6	4.4 ± 3.2	$25.7 \pm 1.9^{g,h)}$	$40.3 \pm 10.1^i)$

a) Maximum plasma level. b) Peak plasma concentration time. c) Apparent half-time of terminal phase. d) Bioavailability (%) = $\frac{AUC_{p.e. \text{ dose i.v.}}}{AUC_{i.v. \text{ dose p.e.}}}$. e) Time for which therapeutic plasma concentration is maintained. f) $p < 0.02$ compared with Rp. 3. g) $p < 0.05$ and h) $p < 0.02$ compared with Hipore 2100. i) $p < 0.02$ compared with Hipore 4050 (0.25 g/2.25 cm²).

of CZP when the transdermal system was applied to rabbits is shown in Fig. 3, along with the time course after direct application of the 2% CZP ointment. The system provided a well sustained plasma concentration of CZP compared with the ointment, and in particular the system with Hipore 4050 gave relatively constant plasma levels for a long time; the therapeutic plasma concentration range (20–70 ng/ml in man)¹²⁾ was sustained for about 26 h. The pharmacokinetic parameters obtained are shown in Table II.

In order to sustain the plasma concentration of CZP for a prolonged period, a system with an increased amount of ointment (0.5 g) and enlarged absorption area (4 cm²) was prepared using Hipore 4050, and applied to rabbits. The plasma CZP concentrations are depicted in Fig. 3. This system sustained a concentration of over 20 ng/ml for about 40 h, with a high bioavailability (37%) and reasonably constant plasma levels.

Anticonvulsant Activity and Plasma Concentration after Application of 2% CZP Ointment and Transdermal Therapeutic System to Rats The time course of plasma concentration of CZP, when the systems (0.12 g, 0.25 cm²) and 2% ointment (0.12 g, 0.25 cm²) were applied to the shaved abdominal skin of rats, are shown in Fig. 4. The plasma concentration profiles obtained were similar to those in rabbits. Sampling points in rats were much fewer than those in rabbits, because the determinations were carried out only to confirm whether or not the levels were in the therapeutic concentration range.

The anticonvulsant activity was estimated after appli-

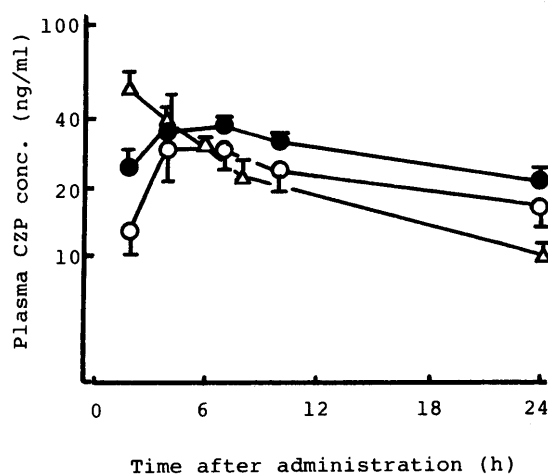


Fig. 4. Plasma Concentration of CZP after Percutaneous Application of Ointment and Membrane-Controlled System to Rats

Each point represents the mean \pm S.D. of 3–4 rats. The applied dose (2% CZP ointment, Rp. 3) was 0.12 g/0.25 cm². Δ , 2% CZP ointment; \bullet , Hipore 4050 system; \circ , Hipore 2100 system.

TABLE III. Anticonvulsant Effect of CZP Ointment and Membrane-Controlled Systems

	Strength of convulsion	Protective score
Placebo	Frank (5/5) ^{a)}	0
Control	Frank (5/5)	0
Ointment (Rp. 3)	None (0/5)	4+
Hipore 2100 system	Slight (1/5)	3+
Hipore 4050 system	None (0/5)	4+

a) Number of rats that died during the experiment.

cation of the systems and 2% CZP ointment to rats. The group given the placebo ointment and the control rats (valproic acid) had severe and persistent convulsion, and all died within 1.5 h. However, the rats given the system with Hipore 4050 and 2% CZP ointment showed no convulsion and no death, and therefore this system was found to be an excellent anticonvulsant device. One of the rats given the system with Hipore 2100 had a frank convulsion, although the remainder showed no convulsion. The anticonvulsant activity and protective score after applying the systems and ointment are summarized in Table III.

The plasma concentrations of CZP when the anticonvulsant activity was measured were 38.4 ± 7.3 ng/ml for the 2% ointment group, 31.9 ± 3.4 ng/ml for the Hipore 4050 system group and 23.8 ± 3.9 ng/ml for the Hipore 2100 system group, which were all in the therapeutic range.

Discussion

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration. Investigation of mechanisms of transdermal drug absorption has led to new approaches in using this route for systemic drug delivery. One of the transdermal systems is the patch, consisting of a reservoir containing the drug and the rate-controlling porous membrane, such as Transderm-V (Ciba-Geigy) and Transderm-Nitro (Ciba-Geigy), and another consists of an adhesive base containing the drug and a copolymer film, such as Frandol tape (Toa Nutrition). It is shown that the delivery rate of scopolamine from Transderm-V is governed by diffusion through the various lamellae of the device and skin. At a steady state, the rate-limiting step is diffusion across the microporous membrane.¹³⁾ Transderm-Nitro also releases nitroglycerin at a constant rate (0.5 mg/cm²·24 h) through the membrane.¹⁴⁾

From the result of the *in vitro* release test, it is evident that the release from the system is the rate-determining step, although the k_r for the drug through the Hipore membrane seems likely to increase in parallel with the mean pore size (Table I).

In the *in vivo* experiments, the transdermal system provided a well sustained plasma concentration of CZP for much longer than 2% CZP ointment. This is probably due to the release rate-limiting capacity of the porous membrane used. However, the Hipore 2100 system, in which mean pore size is smaller (0.23 μ m), yielded relatively lower plasma concentrations and shorter duration, compared with those in the case of the Hipore 4050 system (0.25 g, 2.25 cm²). This may be ascribed to the slower release of the drug through Hipore 2100 and the resultant deficiency of the drug in the absorption site. The fact that the modified Hipore 4050 system (0.5 g, 4 cm²) gave a drug delivery system capable of sustaining the therapeutic plasma concentration for about 40 h suggests that the CZP transdermal system with Hipore 4050 may be exceedingly effective as a controlled-release system, and that a once every two days p.c. administration regimen for epileptic patients may be possible by using the system.

The benzodiazepines have remarkable antiepileptic properties. When anticonvulsant activity of the systems and 2% CZP ointment was measured after injection of pentet-

razole into rats, both systems and the ointment exerted a powerful and complete anticonvulsant activity. The most likely explanation for the high effectiveness is that the plasma concentration of CZP after application of these formulations stayed within the therapeutic concentration range of the drug. Sodium valproate used as the control did not exhibit any anticonvulsant effect. This result may be explained by studies showing that the antiepileptic effect of valproate develops slowly after the start of therapy¹⁵⁾ and the anticonvulsant activity appears to be independent of the serum concentration.¹⁶⁾

In conclusion, the present results lead us to postulate that the drug delivery system based on a microporous membrane, Hipore 4050, gave constant and therapeutic plasma levels of CZP over a long period. The transdermal system has the potential to be an efficient drug delivery system.

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