Research Article

Drug in Adhesive Patch of Zolmitriptan: Formulation and *In vitro/In vivo* Correlation

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Abstract. The objective of the present study was to develop transdermal patch for zolmitriptan, determine its *in vivo* absorption using the rabbit skin. Solvent evaporation technique prepared zolmitriptan patch was settled in two-chamber diffusion cell combined with excised rabbit abdomen skin for permeation study. A sufficient cumulative penetration amount of zolmitriptan ($258.5 \pm 26.9 \mu\text{g}/\text{cm}^2$ in 24 h) was achieved by the formulation of 4% zolmitriptan, 10% Azone, and adhesive of DURO-TAK® 87-4098. Pharmacokinetic parameters were determined via i.v. and transdermal administrations using animal model of rabbit. The results revealed that the absolute bioavailability was about 63%. Zolmitriptan could be detected with drug level of $88 \pm 51 \text{ ng/mL}$ after transdermal administration of 15 min. The *in vivo* absorption curve obtained by deconvolution approach using WinNonlin® program was correlated well with the *in vitro* permeation curve, the correlation coefficient R is 0.84, and the result indicated that *in vitro* skin permeation experiments were useful to predict the *in vivo* performance. In addition, little skin irritation was found in the irritation study. As a conclusion, the optimized zolmitriptan transdermal patches could effectively deliver adequate drug into systemic circulation in short time without producing any irritation phenomenon and worth to be developed.

KEY WORDS: chemical enhancer; drug-in-adhesive patch; *in vitro/in vivo* correlation; pharmacokinetic; zolmitriptan.

INTRODUCTION

Migraine is a very common neurobiological disorder. It is one of the ten most disabling disorders worldwide (1), which attacks as debilitating pain with photophobia, phonophobia, nausea, and vomiting lasting from 4 h even to 3 days (2). These symptoms can produce a significant impact on daily life, which often results in missed work and restricted activity, badly interrupts the quality of life. According to the classification of headache disorders published by IHS (International Headache Society) in 2004 and the guidelines for the diagnosis and management of migraine, triptans are effective and safe for treatment of the level A migraine headache with aura or without aura (3).

Zolmitriptan is a second generation triptan and has been proved to be a highly selective 5-HT_{1B/1D} receptor agonist. Its swallowable tablet, oral disintegrating tablet, and nasal spray in doses of 2.5 and 5.0 mg are available in the market for the treatment of acute migraine. Although triptans provide an excellent therapeutic option for migraine therapy (4), some problems should be noticed. First, rapider onset efficiency and longer action duration are both needed for the termination of

migraine. Because of the short half-life of zolmitriptan in human, multiple dosages of oral administrations are required to resist the recurrence. Oral tablet may also worsen the situation when the migraine attached with significant nausea and vomiting (5). Hence, non-oral medicine administration is necessary to avoid these problems. Advantages of transdermal drug delivery system (TDDS) are the provision of a prolonged period of administration, during which drug levels will be maintained within the therapeutic window. That will offer an extended duration of action and for compounds of short biological half-life and a reduced frequency of dosing. The ease of use of drug-in-adhesive patch will allow for a better patient compliance. For non-invasively improving the skin permeation, the utilization of chemical enhancers was taken into consideration for designing the zolmitriptan patch. Bioavailability of oral administration zolmitriptan is about 40%, which indicates that only a small amount of zolmitriptan is needed to be delivered into circulation system with minimal side effect in treatment of migraine (6). In addition, for acute migraine, short onset effect time is vitally important. One of the major disadvantages of TDDS is long onset effect time for overcoming the skin barrier. For this reason, T_{max} of the *in vivo* study is taken as an important parameter for the evaluation of the optimized patch.

The chemical structure of zolmitriptan is shown in Fig. 1, its physicochemical properties (MW=287.36 g/mol, MP=139–141°) imply that it's suitable for transdermal drug delivery system (7). Although the preparations of zolmitriptan

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Fig. 1. Chemical structure of zolmitriptan

transdermal drug delivery system have been reported in a previous studies (8,9), an *in vivo* evaluation and *in vitro* *in vivo* correlation (IVIVC) have still been lacking for discussing the feasibility of developing the zolmitriptan patches using chemical enhancers.

The objectives of this study were to develop a TDDS containing zolmitriptan and then evaluate *in vivo* permeability with the rabbit model. By achieving these objectives, formulation of zolmitriptan patch was optimized through three main factors: adhesive matrixes, drug loadings, and chemical transdermal enhancers. The optimized formulation patch of zolmitriptan was further investigated with the *in vitro* release study, adhesive properties evaluation, and skin irritation study. For *in vivo* studies, the pharmacokinetic parameters were evaluated by comparing the pharmacokinetic profiles of i.v. administration and transdermal system including optimized formulation (with chemical enhancer patch) and control formulation (without chemical enhancer patch), which indicated that the use of enhancers promised a practical way for treating migraine by TDDS containing zolmitriptan. At last, an IVIVC correlation was established by deconvolution method for speeding up the development of zolmitriptan transdermal patches in follow-up studies.

MATERIALS AND METHODS

Materials

Zolmitriptan (purity $\geq 99\%$) was obtained from Wuhan GPC-China Chemistry Co., Ltd. (Wuhan, China); internal standard rizatriptan benzoate was purchased from National Institutes for Food and Drug Control (Beijing, China); DURO-TAK® 87-4098, DURO-TAK® 87-2677, and DURO-TAK® 87-2852 were purchased from Henkel (Holthausen, Germany); Azone was purchased from Tianjin Bodi Chemical Holding Co., Ltd. (Tianjin, China); N-methyl-2-pyrrolidone (NMP) and Transcutol P were purchased from Beijing Chemical Co., Ltd. (Beijing, China); Oleic acid and Span 80 were purchased from Tianjin Bio Chemical Co., Ltd. (Tianjin, China); Tween 80 was purchased from Beijing Yili Chemical Co., Ltd. (Beijing, China); Isopropyl myristate (IPM) was supplied by China National Medicines Co., Ltd. (Shanghai, China); and

Triethylamine was supplied by Tianjin Bodi Chemical Holding Co., Ltd (Tianjin, China). Potassium dihydrogen phosphate was purchased from Xilong Chemical Industry Incorporated Co., Ltd. (Guangdong, China); Methyl tert-butyl ether (MTBE) was purchased from Sino Pharma Chemical Reagent Co., Ltd. (Shanghai, China); cellophane membrane was purchased from GreenBird; and methanol was HPLC grade and purchased from Hanbon Science and Technology (Jiangsu, China). All other chemicals and solvents were of analytical reagent grade.

Methods

Determination of Zolmitriptan In Vitro

Determination of Zolmitriptan *in vitro* was modified from the previous studies (9,10) The quantitative determination of zolmitriptan was performed by HPLC with Hitachi instruments (Pump L-2130, Auto Sampler L-2200 and UV-VIS Detector L-2420) and Diamonsil C₁₈ reversed-phase column (200×4.6 mm id., 5 μ m; Dikma Technologies, Beijing, China). Mobile phase was a mixture of methanol and distilled water (containing 0.02% potassium dihydrogen phosphate which was adjusted to pH 3.5 by phosphoric acid) at a ratio of 19:81 modified from the previous study. The flow rate was set as 1 mL/min. Twenty microliters of sample was injected for HPLC analysis. The column temperature was maintained at 35°C. The wavelength of the detector was 225 nm. The limit of quantification was 0.5 μ g/mL.

Preparation of Rabbit Skin

Male rabbits weighting 2.0±0.1 kg used in the experiments were purchased from Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The care and use of laboratory animals were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals (11). The rabbits were anesthetized with urethane (20% w/w i.p.), and the abdomen was carefully shaved with a razor after removal of hair by electric clippers. Full thickness skin (epidermis with SC and dermis) was excised from the shaved abdominal site. Subcutaneous fat was

removed using surgical scissors and scalpel. The excised skin was cut into an appropriate size for the two-chamber diffusion cell. The integrity of the skin was carefully checked by microscopic observation; any skin which was not uniform was rejected. The skin was washed immediately with warm phosphate-buffered saline, wrapped in aluminum foil, and stored at -70°C till further use. Right before the experiment, skin was allowed to reach room temperature for at least 1 h. All the frozen skin samples were used within 1 month after preparation.

Preparation of Zolmitriptan Patches

Transdermal patches were prepared by the solvent evaporation technique. Zolmitriptan was dissolved in a minimum amount of ethanol (50 mg/mL). After it was dissolved in ethanol completely, enhancers and pressure sensitive adhesives were added into the drug solution. The mixture was stirred with magnetic bar for 1 h until it became homogeneous, then the mixture was poured on release liner (9744, 3 M, USA). After that, the film was settled at the room temperature (about 25°C) for 10 min and then put into oven set as 50°C for 5 min. The dry PSA film was coated with backing film (9680, 3 M, USA). The constituent weight was calculated by the dry PSA weight.

Preparation of Microscopic Slides for Zolmitriptan Patches

The observation of patch was carried on by optical microscopy (DMBA 450, Motic China Group. Co., Ltd., China). Using the method mentioned above in "Preparation of transdermal patch of zolmitriptan", the drug was dissolved in the solvent and mixed with PSA in different ratio. Mixtures containing different concentrations of zolmitriptan were obtained. The slides were settled in the room temperature for at least 1 week before the observation experiments.

Diffusion Studies

Two-chamber diffusion cell was used in the *in vitro* diffusion study. The opening area of receiver cell was 0.95 cm^2 . The skin was mounted on the receiver cell with the stratum corneum towards outside. The test patch was pasted on the surface of rabbit skin carefully in case of any air bubble between the patch and skin. Two diffusion cells were clamped together patch to patch. Three milliliters of buffer solution was added into receiver cell at the beginning, and the media was stirred by teflon-coated magnetic bar. The jacket chamber was connected with silicone rubber tube full of circulating water insuring that the system was maintained at $32\pm 0.5^{\circ}\text{C}$. Sample collection time points were 2, 4, 6, 8, 10, 12, and 24 h. Two milliliters of receiver solutions were withdrawn and replaced by two (2 mL) of fresh PBS. The collected solution was centrifuged before HPLC analysis.

Data Analysis In Vitro

The cumulative amount in 24 h of zolmitriptan was determined by the concentration and volume of each sampling point (12).

$$Q = \left(\sum_{i=2}^n 2.5C_i - 0.5C_{i-2} \right) / A \quad (i = 2, 4, 6, \dots) \quad (1)$$

Where Q is the cumulative amount penetrated; C_i is the concentration in the receiver compartment at time i ; and A is the effective diffusion area (0.95 cm^2). The cumulative amount penetrated from unit area *versus* time was plotted. For comparison of two groups of data, significance was determined by Students t test. Data were considered significant at a p value of <0.05 .

The activity of penetration enhancers may be expressed in term of an enhancement ratio (ER) (13):

$$ER = \frac{\text{Drug permeation amount used enhancer}}{\text{Drug permeation amount without enhancer}} \quad (2)$$

Partition Coefficient of Zolmitriptan in *n*-octanol/PBS System

A drug solution of $100.0\text{ }\mu\text{g/mL}$ was prepared in *n*-octanol, 2.0 mL of this solution was taken in separating funnel and vortexed with an equal volume of phosphate buffer of pH 7.4 for 0.5 h and allowed for 2 h for standing. Then, the aqueous phase and organic phase were collected separately and centrifuged at 2000 rpm. Both phases were analyzed by HPLC. Partition coefficient was calculated by taking the ratio of the drug concentration in *n*-octanol to drug concentration in aqueous phase.

Patch Thickness and Drug Content Uniformity

The thickness of each patch was measured three times in three different points excluded the back membrane and release liner thickness with Digimatic Micrometer (IP 65, Mitutoyo, Japan), and then average value was taken as the final thickness. For drug content uniformity, the patch was cut into 3 cm^2 and added into beaker of 50 mL filled with 50% of ethanol and 50% of methanol. The sample was under ultrasonic processing twice, first for 30 min and second for 15 min. Then, the solution was moved to volumetric flask (100.0 mL), the content was filtered with filter paper and injected into HPLC for content determination.

Skin Irritation Test

Skin irritation test was evaluated by the Draize method (14). Six healthy rabbits were used in this test. The rabbits were shaved at abdominal skin both left and right. Aqueous solution of lauryl sodium sulfate at the concentration of 10% *w/v* was used as standard irritant. Patches containing zolmitriptan of 3.0 cm^2 were used as test patches. Ten percent of lauryl sodium sulfate solution was applied on the left abdominal skin, and the patch was placed on opposite site, then the patches were removed after a period of 24 h with the help of alcohol swab. Scores were graded after the transdermal patch administration at 24, 48 (patch removed), and 72 h (patch removed) to observe the development of erythema and edema for each rabbit. When the control was used, the obtained scores were subtracted from the primary irritation index.

The category of irritation was scored on the basis of the mean primary irritation index as follows:

- 0.0–0.4, negligible response;
- 0.5–1.9, slight response;
- 2.0–4.9, moderate response;
- 5.0–8.0, severe response.

Determination of Zolmitriptan In Vivo

Determination of zolmitriptan was obtained using Waters Instruments with fluorescence detection (Waters e2695 Separation Module, Waters 2475 Multi λ Fluorescence Detector). Diamonsil C₁₈ reversed-phase column (200 $\times$ 4.6 mm id., 5 μ m; Dikma Technologies, Beijing China). The mobile phase was a mixture of methanol and distilled water (containing 0.05% triethylamine, adjusting to pH 3.5 with phosphoric acid) at a flow rate of 1.0 mL/min. Fluorescence detection was performed at 225 nm (excitation) and 360 nm (emission) (15). The linearity was checked in the interval of 8–400 ng/mL ($y=339.96x-5.06$; $R^2=0.9978$), and the limit of quantification was 8 ng/mL.

Pharmacokinetic Study

Sample Collection

Male rabbits (weighting 2.0 $\pm$ 0.1 kg) were used in the *in vivo* pharmacokinetic analysis. During the experiment, rabbits were given access to food and water freely. The weight of each rabbit was measured right before the experiment.

The experiment was designed as crossover trial, which meant that the same rabbit was used twice in the experiments including i.v. injection and transdermal administration with optimized patches. Four male rabbits were used i.v. injection of 0.90 mg/kg (dissolved in saline containing 2% alcohol) study and then zolmitriptan patch transdermal study. Patches without chemical enhancer were administrated to another four of rabbits. About 0.5 mL blood sample was collected from another marginal ear vein and placed in dried heparinized tube. One hundred fifty microliters of plasma was taken for sample preparation.

In the i.v. administration, zolmitriptan solution was injected via marginal ear vein; the collected time points were 0.08, 0.17, 0.25, 0.50, 1.50, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, and 12.0 h after the i.v. bolus administration.

Before the patches were applied, abdominal site of the rabbit was shaved and cleaned with saline. The skin of the rabbits was checked carefully in case of any skin breakage. Following transdermal administration, each rabbit was given patches containing zolmitriptan 7.64 mg. The sample collected time points were 0.25, 0.5, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 28.0, and 32.0 h. The patches were removed from rabbits after 24 h with the help of alcohol swab.

Plasma samples were separated immediately by centrifugation at 4000 rpm for 5 min at room temperature and stored at -20°C until analysis.

Sample Preparation

Plasma sample preparation method was modified from the previous literatures (15). Twenty microliters of IS solution (rizatriptan benzoate, 5 μ g/mL in 10% methanol solution) and 20 μ L of methanol were added into 150 μ L plasma sample in the centrifuge tube of 2 mL. Then, twenty microliters of NaOH solution (1.0 mol/L) was added, the mixture was vortex-mixed for 30 s and extracted with 1 mL of MTBE by shaking for 5 min. After that, the mixture was settled for 10 min until the organic and aqueous phases were separated.

The organic phase was transferred into another tube and evaporated under the stream of nitrogen at the room temperature. One hundred fifty microliters of mobile phase was added and vortex-mixed for 1 min. Twenty microliters of samples were injected into the HPLC by the auto-sampler for the analysis. The extraction recovery was $88.31\pm7.74\%$ for zolmitriptan.

Pharmacokinetic Analysis

Plasma concentration profiles of i.v. injection rabbits were individually fitted to pharmacokinetic non-compartmental i.v.-bolus input model and extravascular input model using WinNonLin® (version 5.2.1, Pharsight Corporation, Mountain View, CA). All the pharmacokinetic parameters were calculated by this software.

Absolute bioavailability (F) was calculated from the following equation:

$$F = \frac{AUC_{trans}}{AUC_{i.v.}} \times \frac{\text{dose}_{i.v.}}{\text{dose}_{trans}} \quad (3)$$

AUC of transdermal administration was expressed as AUC_{trans} . The steady-state plasma concentration of zolmitriptan after the application of patches was calculated from the equation (16): $C_{ss}=AUC_{0-T}/T$, T is the administrations time of 24 h. Data are expressed as the mean $\pm$ standard deviation (S.D.).

In Vitro/In Vivo Correlation

For the establishing of *IVIVC*, *in vivo* permeation data of zolmitriptan patch were obtained plasma concentration by deconvolution method and the metabolized part is adjusted by the i.v. data. The deconvolution process can be expressed as the following equation:

$$R(t) = \int_0^t I(\theta)W(t-\theta)d\theta \equiv I(\theta)*W(\theta) \quad (4)$$

Where $R(t)$, I , and W are the plasma concentration as a function of time, the input into the system (*in vitro* skin permeation data), and the weight function (i.v. data), respectively, and $*$ is the convolution operator. Convolution was done by solving this formula with respect to $R(t)$ given $I(t)$ and $W(t)$, whereas deconvolution was done by solving the following equation for $I(t)$ given $R(t)$ and $W(t)$.

$$I(t) = R(t) // W(t) \quad (5)$$

Where the symbol $//$ denotes the deconvolution operation. Then the calculated *in vivo* absorption time profile is connected to the *in vitro* permeation profile (17). With the help of WinNonlin® with *IVIVC* toolkit program and correlation coefficients were examined for significance ($p<0.05$) using Student's test.

RESULTS AND DISCUSSION

The Optimization of Zolmitriptan Transdermal Patch

The results of optimization process of transdermal zolmitriptan patches including adhesive matrix, drug loading,

and permeation enhancers are shown in Table I. The cumulative permeation profiles in different adhesive matrix were first investigated, including DURO-TAK® 87-4098, DURO-TAK® 87-2677, and DURO-TAK® 87-2852. The results are shown in Table I. Significantly higher flux was obtained from the adhesive matrix of DURO-TAK® 87-4098 ($p < 0.05$). Acrylic PSAs offered advantages of good compatibility with a wide range of drugs, ease of processing, they were resistant to oxidation because of the saturated hydrocarbon, did not require the addition of stabilizers, which could cause skin irritation (18). According to the study of Subedi et al.(8), higher permeation amount was obtained using Duro-Tak® 87-2510, which was hydroxyl acrylic pressure sensitive adhesive, the final penetration amount was over 200 $\mu\text{g}/\text{cm}^2$ in 4% drug loading without any transdermal enhancer. But according to our preliminary experiments, it was hard to dissolve zolmitriptan into Duro-Tak® 87-2510 without the help of ethanol in 2% drug loading and 4% drug loading became milky status; it could be concluded that the compatibility between zolmitriptan and Duro-Tak® 87-2510 was not well enough. From the aspect of product stability, crystallization potential might influence the stability of transdermal patch of future product. Therefore, Duro-Tak® 87-2510 was not taken into consideration in the formulation screening; further studies were carried out based on DURO-TAK® 87-4098 adhesive matrix.

Drug loading is an important factor which influences drug permeation ability. In order to investigate the effect of drug loading, 2, 4, and 6 of zolmitriptan concentration were selected; higher permeation amounts were achieved from 6% drug loading and there was no significant difference between 4 and 6% drug loading ($p > 0.05$). In order to observe crystallization of zolmitriptan in adhesive matrix further, three different formulations slips were prepared then observed under the microscope. Before the observation, they were settled in room temperature at least 1 week ensuring that crystalline separated out from the supersaturated matrix. The results are shown in Fig. 2. Crystallization of zolmitriptan was easily observed in the micrograph of the slide containing 6% drug loading, not found in 2 and 4% patches. In regard to a drug-in-adhesive

patch, drug was dissolved in the adhesive matrix directly. The patches maximum drug loading was the saturation solubility in adhesive matrix, especially in the method of solvent evaporation technique. When the solvent evaporated from the mixture, supersaturated matrix would be produced. Though supersaturated was used to obtain greater penetration amount in some studies (19), such a supersaturated matrix is the reason of crystallization, which would make the patch unstable (20). According to Fick's First law, the permeation across the skin was proportional to the dissolved drug concentration in the patches, and the crystalline phase was inactive for the permeation (21). This result was in accordance with the study of Subedi et al.(8) though in different type acrylic pressure sensitive adhesive, there was no significant difference among 4% drug loading to 10%, which proved that crystallization of zolmitriptan in matrix had little contribution to the permeation amount. Increasing drug loading was not suitable way to improve transdermal permeation amount. Hence, 4% drug loaded patches was selected for the next studies.

Since breaking through the barrier of stratum corneum is the most difficult part for transdermal process, several kinds of physical and chemical methods were made use of in TDDS. Chemical enhancers were most widely used because of the ease use, relatively stable in patch and low-cost (22). Different chemical permeation enhancers were added into the patches to investigate their effects. Table I gives the chemical permeation enhancers added in the patches based on the adhesive matrix of DURO-TAK® 87-4098 and 4% zolmitriptan drug loading.

Azone had significant effect to 4% zolmitriptan single patch ($p < 0.05$), and it contributed an *ER* value of 1.51 at the concentration of 5%. Other transdermal enhancers did not exhibit any significant difference by comparison with control group. Therefore, Azone was selected as zolmitriptan transdermal patch enhancer for the next studies. Concentrations of Azone were optimized to investigate the maximum effect of their enhancement within concentration range of 2.5~10%. The results are shown in Table I. Azone (10%) in the patch exhibited the best enhancement effect with *in vitro ER* of 2.56. At last, the optimized formulation patch of zolmitriptan cu-

Table I. The Results of *In Vitro* Formulation Screening

Influencing factors	Drug content	Adhesive matrix (DURO-TAK®)	Chemical Enhancers	Q_{24} ($\mu\text{g}/\text{cm}^2$)	ER
PSA ¹	4%	87-4098	–	101.14 $\pm$ 21.83	–
	4%	87-2677	–	36.95 $\pm$ 6.93	–
	4%	87-2852	–	23.62 $\pm$ 0.41	–
Drug content	2%	87-4098	–	68.94 $\pm$ 1.62	–
	6%	87-4098	–	127.98 $\pm$ 4.41	–
Transdermal enhancer	4%	87-4098	5% Azone	153.11 $\pm$ 7.01	1.51
	4%	87-4098	5% Span	130.22 $\pm$ 6.12	1.29
	4%	87-4098	5% Tween	119.93 $\pm$ 29.03	1.19
	4%	87-4098	5% Transcutol P	118.98 $\pm$ 28.39	1.18
	4%	87-4098	5% NMP ²	117.13 $\pm$ 13.06	1.16
	4%	87-4098	5% IPM ³	91.44 $\pm$ 8.25	0.90
	4%	87-4098	5% oleic acid	52.26 $\pm$ 17.71	0.52
Transdermal enhancer content	4%	87-4098	10% Azone	258.51 $\pm$ 26.86	2.56
	4%	87-4098	2.5% Azone	113.34 $\pm$ 24.19	1.12

Data are given as average $\pm$ SD ($n=3$)

PSA pressure sensitive adhesive, NMP *N*-methyl-2-pyrrolidone, IPM Isopropyl myristate

Fig. 2. Crystallization of zolmitriptan on slides in different ratios. **a** blank; **b** 2%; **c** 4%; **d** 6% (40×10)

mulative amount penetrated increased to $258.51 \pm 26.86 \mu\text{g}/\text{cm}^2$, with a flux of $12.28 \pm 1.12 \mu\text{g}/\text{h}/\text{cm}^2$ in 24 h. According Ren et al. (23), there was functional group of carboxyl in DURO-TAK® 87-2677 and DURO-TAK® 87-2852, which may lead to interaction with amine group of zolmitriptan (24). This was the reason of low penetration rate from two kinds of PSA. Azone was the first molecule specifically designed as a skin penetration enhancer, and widely used in the permeation enhancement of steroids, antibiotics, and antiviral agents (25). The enhancer effect of Azone depended predominantly on two parameters, the lipophilicity of the compound assayed and the concentration of Azone employed (26). Physicochemical property of zolmitriptan was determined by partition coefficient study. In this study, *n*-octanol and *in vitro* fluid (phosphate buffer solution, pH 7.4) were considered to be standard system for determining the drug partition coefficient between skin and *in vitro* fluid (27). The logarithmic value of partition coefficient ($\log P_{O/PBS}$) was found to be -0.85 ± 0.08 ($n=4$) in partition study. It was concluded that zolmitriptan was more hydrophilic in the system of PBS and *n*-octanol. As we knew, stratum corneum was described by Barry with the theory of “protein bricks in a lipid mortar”, which meant that for the hydrophilic drug, it diffuses relatively into the aqueous protein bricks easily. However, in order for it to traverse completely through the skin, it must move across the lipophilic mortar substance to another region of protein. Therefore, we concluded that the rate-limiting step for zolmitriptan percutaneous absorption was the lipophilic region of stratum corneum. According to the previous literature (28,29), Azone does not appear to interact with proteins; it partitioned directly into the lipid bilayer and disrupts it, making the lipids more fluid and flexible. Hydrophilic drugs, i.e., zolmitriptan, will

benefit from the use of Azone, which will enhance the permeability of zolmitriptan with the *ER* of 2.56 with 10% Azone, and according to the irritation test result, which is showed in Table II, 10% Azone containing patch did not exhibit any irritation phenomenon on the rabbit skin compared with standard irritant group. Skin irritation phenomenon depends on the release amount that Azone released from the PSA layer. According to the study of Qvist et al. (30), Azone was a lipophilic transdermal enhancer and had good compatibility with the pressure sensitive adhesive, and it had a lower release rate from the acrylic pressure sensitive adhesive independent to the type of adhesive. In this study, acrylic type pressure sensitive adhesive Duro-Tak® 87-4098 was used as adhesive matrix, and it could be concluded that not all Azone could be released from the matrix in administration duration.

As a result, the final formulation of zolmitriptan transdermal patch could be based on the formulation that containing 4% zolmitriptan, 10% Azone DURO-TAK® 87-4098 as adhesive matrix. This formulation was used for next *in vivo* studies. The thickness and drug content uniformity of the developed patch were determined to ensure the quality consistency between three different batches. The results are shown in Table III.

Table II. Results of Skin Irritation Test

	24 h	48 h	72 h
Optimized formulation	0.1 ± 0.1	0.0	0.0
Standard irritant	6.8 ± 0.8	7.3 ± 0.8	7.8 ± 0.4

Data are given as average $\pm$ SD ($n=6$)

Table III. Patch Thickness and Content Uniformity of Zolmitriptan Patch

Batches	Thickness* (μm)	Drug content (%)
1	103 $\pm$ 4	97.3 $\pm$ 3.1
2	98 $\pm$ 3	94.8 $\pm$ 2.0
3	108 $\pm$ 3	103.4 $\pm$ 4.5

*Back membrane and release liner thickness are not included
Data are given as average $\pm$ SD ($n=3$)

Pharmacokinetic Study and In Vitro/In Vivo Correlation

A total of four rabbits were chosen both in the case of i.v. injection and transdermal administration. Since zolmitriptan transdermal patch was designed as daily drug delivery system, 24 h *in vivo* drug administration study was carried out. Pharmacokinetic study of 4% of zolmitriptan containing patch without enhancer (control) was also carried out as blank group. The mean plasma concentration–time profiles and pharmacokinetic parameters of zolmitriptan after i.v. and transdermal administration are shown in Fig. 3 and Table IV, respectively. After i.v. injection of zolmitriptan, the disposition was rapidly in the first 10 min and the mean plasma concentration was minimal at 12 h. AUC_{0-t} of 316 $\pm$ 34 h ng/mL and MRT_{0-t} of 1.33 $\pm$ 0.54 h were achieved, respectively.

Figure 3 also shows the plasma profile of zolmitriptan after transdermal administration of patch with Azone (optimized) and without transdermal enhancer (control). In the first 2 h, the absorption rate of zolmitriptan had showed significant difference between the two kinds of patches. A higher absorption rate was obtained from patches containing Azone, for which zolmitriptan was detected in the blood stream at 15 min and drug levels were 87 $\pm$ 51 and 116 $\pm$ 10 ng/

mL at time point of 0.5 and 1.0 h, respectively. C_{max} of 136 $\pm$ 13 ng/mL was obtained at T_{max} of 1.63 $\pm$ 1.11 h. Though zolmitriptan was detected in the blood stream at 15 min from patches without Azone, the plasma concentration was about only 21 $\pm$ 7 ng/mL, which was much less than the patches with Azone. C_{max} of patches without Azone was about 53 $\pm$ 9 ng/mL at T_{max} of 1.88 $\pm$ 0.85 h, which was almost one third of C_{max} of patch with Azone. It was noted that zolmitriptan in the transdermal patches could rapidly enter into the systemic circulation and achieve the maximum concentration with the help of Azone in the first 1.63 $\pm$ 1.11 h, which indicated that the onset of action achieving from TDDS was acceptable (2). This effect was closely associated with the permeation enhancement of Azone, which will enter into skin and create disorder (22). In the first 4 h, plasma concentration of zolmitriptan was maintained at a relatively high level, which meant that adequate zolmitriptan was delivered into systemic circulation in resisting the eliminating. AUC_{0-t} was 838 $\pm$ 73 h ng/mL, after dose normalization, the absolute bioavailability of transdermal patch was 63%, AUC_{0-t} of patch without Azone was 330 $\pm$ 43 h ng/mL with absolute bioavailability of only 25%. The effect of Azone was obviously important in enhancing zolmitriptan penetration amount. According to the study of Seaber et al. (31), incomplete absorption and first-pass metabolism played part in determining the low oral bioavailability of zolmitriptan (40%), transdermal drug delivery system had an advantage in avoiding first-pass metabolism, delivering drug into system circulation directly without any first-pass metabolism phenomenon, which improved the bioavailability up to 63%. MRT_{0-t} of transdermal patch was prolonged to 5.69 $\pm$ 1.27 h, in view of the longer MRT_{0-t} compared with i.v. injection (1.33 $\pm$ 0.54 h), the enhancement of treatment effect duration was practicable.

Data of *in vivo* indicated that adequate amount of zolmitriptan was delivered into rabbit systemic circulation

Fig. 3. Plasma concentration–time profiles of zolmitriptan (square dot represents optimized formulation, the circle dot represents control patch without enhancer and the triangle represents intravenous injection). Data are given as average $\pm$ SD ($n=4$)

Table IV. Pharmacokinetic Parameters of Zolmitriptan after Intravenous Injection of Zolmitriptan Via Marginal Ear Vein, Transdermal Administration of Control Formulation and Optimized Formulation

Parameters	i.v.	Transdermal (Control)	Transdermal (Optimized)
$T_{\max}$ (h)	–	1.88±0.85	1.63±1.11
$C_{\max}$ (ng/ml)	–	53±9	136±13
AUC_{0-t} (h ng/mL)	316±34	330±43	838±73
Cl (mL/h)	5054±672	–	–
$AUMC_{0-t}$ (h ² ng/mL)	377±101	1678±379	4812±1393
C_{ss} (ng/mL)	–	14±2	35±3
MRT_{0-t} (h)	1.33±0.54	5.04±0.55	5.69±1.27
ER <i>in vivo</i>	–	–	2.54

Data are given as average±SD (n=4)

and onset effect of the patch was able to achieve within about half an hour, which as a most important factor in the treatment of the acute migraine. Azone was the main factor that enhanced the delivery of zolmitriptan into systemic circulation. The effect of Azone was tested through *in vivo* studies by comparing the penetration of optimized zolmitriptan patches (with Azone) and control patches (without Azone). *In vivo* ER value of 2.54 was achieved using AUC value instead of Q_{24} value *in vitro*, which is showed in Table IV. It was almost the same as *in vitro* ER value of 2.56, which is showed in Table I, the result demonstrated that Azone achieves the same enhancement rate both *in vitro* and *in vivo*.

In vitro-in vivo correlation is the relationship between an *in vitro* parameter and an *in vivo* parameter. *In vitro* measurement should predict *in vivo* performance of the formulation. For zolmitriptan patch preparations, *in vitro* measurement is the drug penetration through the skin and *in vivo* measurement is the drug concentration in plasma. The purpose of the

correlation establishing is to predict *in vivo* performance by *in vitro* measurement. As present in Fig. 4, the deconvoluted absorption profile *in vivo* of different rabbits is in agreement with the observed absorption profile *in vitro*, and the correlation coefficient is 0.84 ($p<0.05$), the result of *IVIVC* relationship demonstrated that *in vitro* permeation experiments can be utilized to optimize formulations in further studies to develop commercial products.

CONCLUSIONS

In present study, an optimized patch was developed for transdermal delivery of zolmitriptan. On the basis of *in vitro* and *in vivo* characterization, it was concluded that zolmitriptan could be administered transdermally through drug-in-adhesive type TDDS. The absolute bioavailability of 63% and MRT_{0-t} of 5.74±0.83 h and $T_{\max}$ of 1.63±1.11 h demonstrated that satisfactory amount of zolmitriptan could be delivered into blood in a short timeframe with the assist of Azone; easy use of drug-in-adhesive patch without any irritation phenomenon would also improve the migraineur compliance. As a conclusion, development of an effective transdermal drug delivery system for zolmitriptan may be a feasible strategy for the treatment of migraine.

Conflict of Interest The authors report no conflicts of interest.

REFERENCES

1. Antonaci F, Dumitrache C, De Cillis I, Allena M. A review of current European treatment guidelines for migraine. *J Headache Pain*. 2010;11(1):13–9.
2. Silberstein SD. Migraine. *Lancet*. 2004;363(9406):381–91.

Fig. 4. Drug penetration profile of zolmitriptan patch *in vivo* (dashed line) (n=4) and *in vitro* (square dot line) (n=3)

3. International Headache Society. The International classification of headache disorders. Cephalalgia. 2004;24(s1):9–160.
4. Saxena PR, Tfelt-H P. Success and failure of triptans. J Headache Pain. 2001;2:3–11.
5. Rizzoli PB. Acute and preventive treatment of migraine. Am Acad Neurol. 2012;18(4):764–82.
6. Moeller FG, Bjork JM, Dougherty DM, Van de Kar LD, Marsh DM, Swann AC. Low dose zolmitriptan as a 5-HT neuroendocrine challenge agent in humans. Psychoneuroendocrinology. 2000;25(6):607–18.
7. Naik A, Kalia YN, Guy RH. Transdermal drug delivery: Overcoming the skin's barrier function. Pharm Sci Technol Today. 2000;3:318–26.
8. Subedi RK, Ryoo JP, Moonb C, Choi HK. Influence of formulation variables in transdermal drug delivery system containing zolmitriptan. Int J Pharm. 2011;419(1–2):209–14.
9. Patel SR, Zhong H, Sharma A, Kalia YN. Controlled non-invasive transdermal iontophoretic delivery of zolmitriptan hydrochloride *in vitro* and *in vivo*. Eur J Pharm Biopharm. 2009;72:304–9.
10. Saka C. Review of analytical methods for identification and determination of triptans. Crit Rev Anal Chem. 2009;39:32–42.
11. National Research Council (US) Institute for Laboratory Animal Research. Guide for the care and use of laboratory animals. Washington D.C: National Academy Press (US); 1996.
12. Sun Y, Fang L, Zhu M, Li W, Meng P, Li L. A drug-in-adhesive transdermal patch for S-amlodipine free base: *In vitro* and *in vivo* characterization. Int J Pharm. 2009;382:165–71.
13. Chen Y, Wang J, Cun D, Wang M, Jiang J, Xi H, *et al.* Effect of unsaturated menthol analogues on the *in vitro* penetration of 5-fluorouracil through rat skin. Int J Pharm. 2014;443:120–7.
14. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther. 1944;82:377–90.
15. Chen J, Jiang XG, Jiang WM, Mei N, Gao XL, Zhang QZ. High-performance liquid chromatographic analysis of zolmitriptan in human plasma using fluorescence detection. J Pharm Biomed. 2004;35:639–45.
16. Valiveti S, Hammell DC, Paudel KS, Hamad MO, Crooks PA, Stinchcomb AL. *In vivo* evaluation of 3-alkyl ester transdermal prodrugs of naltrexone in hairless guinea pigs. J Control Release. 2005;102:509–20.
17. Sun L, Cun D, Yuan B, Cui H, Xi H, Mu L, *et al.* Formulation and *In Vitro/In Vivo* correlation of a drug-in-adhesive transdermal patch containing Azasetron. J Pharm Sci. 2012;101:4540–8.
18. Tan HS, Pfister WR. Pressure-sensitive adhesives for transdermal drug delivery systems. Pharm Sci Technol Today. 1999;2(2):60–9.
19. Moser K, Kriwet K, Kalia YN, Guy RH. Enhanced skin permeation of a lipophilic drug using supersaturated formulations. J Control Release. 2001;73:245–53.
20. Jain P, Banga AK. Inhibition of crystallization in drug-in-adhesive-type transdermal patches. Int J Pharm. 2010;394:68–74.
21. Kim JH, Choi HK. Effect of additives on the crystallization and the permeation of ketoprofen from adhesive matrix. Int J Pharm. 2002;236:81–5.
22. Benson HA. Transdermal drug delivery: Penetration enhancement techniques. Curr Drug Deliv. 2005;2(1):23–33.
23. Ren C, Fang L, Ling L, Wang Q, Liu S, Zhao L, *et al.* Design and *in vivo* evaluation of an indapamide transdermal patch. Int J Pharm. 2009;370:129–35.
24. Morimoto Y, Kokubo T, Sugibayashi K. Diffusion of drugs in Acrylic-type pressure-sensitive adhesive matrix. II. Influence of interaction. J Control Release. 1992;18:113–22.
25. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta. 2009;1788:2362–73.
26. Diez-Sales O, Watkinson AC, Herráez-Domínguez M, Javaloyes C, Hadgraft J. A mechanistic investigation of the *in vitro* human skin permeation enhancing effect of Azone®. Int J Pharm. 1996;129:33–40.
27. Singh UV, Pandey S, Udupa N. Preparation and evaluation of flurbiprofen and diclofenac sodium transdermal films. Indian J Pharm Sci. 1993;54:145–7.
28. Phillips CA, Michniak BB. Transdermal delivery of drugs with differing lipophilicities using Azone analogs as dermal penetration enhancers. J Pharm Sci. 1995;84(12):1427–33.
29. Jampilek J, Brychtova K. Azone analogues: Classification, design, and transdermal penetration principles. Med Res Rev. 2012;32:907–47.
30. Qvist MH, Hoeck U, Kreilgaard B, Madsen F, Frokjaer S. Release of chemical permeation enhancers from drug-in-adhesive transdermal patches. Int J Pharm. 2002;231:253–63.
31. Seaber EJ, Peck RW, Smith DA, Allanson J, Hefting NR, van Lier JJ. The absolute bioavailability and effect of food on the pharmacokinetics of zolmitriptan in healthy volunteers. Br J Clin Pharmacol. 1998;46(5):433–9.