Acta Poloniae Pharmaceutica – Drug Research, Vol. 65 No. 4 pp. 481–486, 2008 ISSN 0001-6837
Polish Pharmaceutical Society

PHARMACEUTICAL TECHNOLOGY

EX VIVO AND *IN VIVO* EVALUATION OF TRANSDERMAL FORMULATION OF TRAZODONE HYDROCHLORIDE

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Abstract: The aim of the present investigation is to evaluate the biopharmaceutical behaviors of the matrix patch containing trazodone hydrochloride (TZN) following transdermal administration in rabbits. The *ex vivo* skin permeation study was performed using Keshary-Chien glass diffusion cell and mouse epidermis with intact stratum corneum as membrane. The phosphate buffer of pH 7.4 was used as receptor solution at 37°C. TZN patch was applied to the inner pinna skin of the rabbit. TZN transdermal absorption from patch was compared to that from peroral TZN solution in rabbit. A steady-state skin permeation rate of 134.09 ± 2.49 μg/cm^2/h was achieved from the matrix patch across mouse epidermis after an initial lag time of ~3.5 h. The steady-state transdermal TZN concentration of 2.3 μg/mL was achieved in rabbit from the matrix patch after an initial lag time of ~2 h. The C_{max} of peroral TZN solution was calculated to be 5.84 μg/mL at a T_{max} of 2 h indicating its rapid absorption compared to the transdermal administration with a T_{max} of 5 h. The *ex vivo* and *in vivo* biopharmaceutical parameters were in good agreement with respect to steady-state plasma TZN concentration and lag time. The plasma level of TZN following transdermal application could be maintained for 24 h. The transdermal dose achieved a much higher steady-state blood concentration in rabbit compared with the effective blood concentration in human. The observed steady-state blood concentration may appear to be within the expected therapeutic range in human with a higher clearance value compared to that in rabbit.

Keywords: trazodone hydrochloride, matrix patch, *ex vivo*, *in vivo*, Keshary-Chien diffusion cell, rabbit model

Trazodone hydrochloride (TZN), a serotonin uptake inhibitor, is used in the treatment of moderate to severe depression in schizophrenic patients. The undesirable side effects of TZN administered orally can be offset by using the transdermal route, which attenuates the fluctuating TZN levels (52 – 81%) resulting from oral therapy. The plasma half-life of TZN is about 6 h, which requires frequent dosing necessary to maintain the therapeutic blood level (0.75 μg/mL) for a long-term treatment (1-3) For the treatment of depression, the dose of TZN for adults ranges from 150 – 600 mg administered as one to four tablets a day. The dose and frequency may cause enhanced drug related side effects and may pose compliance problems. The transdermal route of administration is capable of controlled delivery of TZN avoiding the higher dose related major side effects and thereby improving patients' compliance. Das et al. (4) reported its feasibility for transdermal delivery. The matrix type monolithic device has recently been developed in our laboratory for the transdermal delivery of TZN. The device has been shown to deliver TZN transdermally at a zero-order rate over a 24 h period across mouse and human cadaver epidermis *in vitro* (5, 6).

The present work is the first *in vivo* work in which the biopharmaceutical behaviors of the developed formulation following transdermal administration in rabbits is reported.

EXPERIMENTAL

Materials

Trazodone hydrochloride (ICN Pharmaceuticals, Inc., Eschwege, Germany), Eudragit RL100 and RS 100 (gift samples from Rohm Pharma, Darmstadt, Germany), isopropanol, acetone, sodium chloride, potassium dihydrogen orthophosphate (Ranbaxy Laboratory, New Delhi, India), triethylcitrate (E. Merck, Darmstadt, Germany), disodium hydrogen orthophosphate (Qualigens Fine Chemicals, Mumbai, India), fennel oil (Allied Chemical Corporation, Vadodara, India) were used.

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Animals

The Institutional Animal Ethics Committee of Dibrugarh University, India approved all experiments with animals. The male albino mice of 6-7 weeks old (M/S Ghosh Enterprises, Kolkata, India) and the male albino rabbits of 1.5-2.0 kg (M/S Chakraborty Enterprises, Kolkata, India) were used for the experiments. Animals were allowed to be acclimatized for a period of 2 weeks in our laboratory environment prior to the study and maintained under controlled conditions of temperature as well as humidity and had free excess to water and food. The principles of Laboratory Animal Care (NIH, 1985) were followed and instructions given by our institutional animal ethical committee were maintained throughout the experiment.

METHODS

Preparation of TZN transdermal devices

The method of preparation of transdermal films was based on that developed by Das et al. (6). In brief, the method is as follows: TZN at 10% w/w (based on polymer weight) was dispersed in the polymeric solution (10% w/v) of the mixture of Eudragit RL 100 and RS 100 (1 : 3) in a binary mixture of isopropanol and acetone (3:2, v/v). Triethylcitrate was used as plasticizer in the film formulation at 13% w/w (based on polymer weight) level. Fennel oil at 10% w/w (based on polymer weight) level was added as a penetration enhancer to the formulation after dissolving the polymers. The mixture was cast over backing membrane of Eudragit NE 30D previously formed in aluminum Petri dish (13 cm^2) and dried under controlled conditions (temperature 32^oC, RH 45%) for 24 h to reach a stable drug-polymer matrix. The thin and opaque film formation indicates that the solvent for polymer got completely evaporated in 24 h whereas triethylcitrate and fennel oil remained in the drug-polymer matrix. The presence of fennel oil in the polymer matrix can be confirmed by the penetration data from the skin permeation study in the previous report (5). The permeation enhancing effect of fennel oil was confirmed by the *in vitro* skin permeation study where TZN was delivered from control formulation (without penetration enhancer) across mouse epidermis with pretreatment with fennel oil and through the skin with no pretreatment. An enhancement ratio of 9.25 was observed in pretreatment experiment as compared with no pretreatment. When TZN was delivered from the formulation containing fennel oil across mouse epidermis, the value was 5.22 as compared to the control formulation. The inclusion of fennel oil into the transdermal device showed lower enhancing factor as compared to the pretreatment experiment. It can be explained by the fact that the fennel oil was delivered directly on the skin surface in pretreatment experiment whereas it must diffuse out of the device containing fennel oil to reach the skin surface for its action. The drug content in the transdermal films was measured by the method reported by Sutinen et al. (7). Approximately 99.21 ± 2.34% of the theoretical amount added was found in the prepared transdermal films.

***Ex vivo* study**

The *ex vivo* skin permeation study was performed using Keshary-Chien glass diffusion cell and mouse epidermis with intact stratum corneum (SCE) as a membrane (5). The method of SCE preparation was based on that developed by Kligman and Christophers (8) and Swarbrick and Brown (9). The film sample was fixed on the skin sample previously fixed in between the donor and receptor compartment of the diffusion cell. The stratum corneum side of the skin was kept in intimate contact with the release surface of the transdermal film and dermal side facing the receptor solution. The receptor solution (phosphate buffer of pH 7.4) at 37^oC was introduced into the stirred receptor compartment that was maintained at 37^oC by a circulating water bath (NSW-133, NSW, India) using an aquarium liquid filter (LifeTech AP 1200). Samples from the receptor compartment were withdrawn at predetermined time intervals and immediately replaced by an equal volume of fresh buffer solution maintained at 37^oC. Initial experiments confirmed the maintenance of sink condition by this procedure (5). The samples were then analyzed spectrophotometrically at 246 nm using Hitachi U-2001 UV-VIS spectrophotometer. The concentrations of TZN in the samples were calculated using regression equation:

$$(\text{absorbance} = -0.004 + 0.0264 \times \text{concentration}, \quad r^2 = 0.999)$$

of the calibration curve of TZN in phosphate buffer of pH 7.4 and corrected to compensate the loss due to sample withdrawal, using the equation proposed by Hayton and Chen (10).

***In vivo* study**

The male healthy rabbits weighing 1.5 to 2 kg were chosen for the study. The rabbits were fasted overnight but water was allowed *ad libitum*. The rabbits were divided into four groups of three rabbits each. The rabbits were kept in cages with husk bedding. On the next morning TZN (50 mg) was admin-

istered orally as a solution in distilled water via a feeding tube (~ 5 mL as a bolus) to group I. The food was allowed at 4 h after drug administration. The blood samples were taken at 0, 0.5, 1, 2, 3, 4 and 8 h after administration. Group II rabbits received 1 mL of intravenous solution of TZN equivalent to 5 mg/kg body weight via the marginal ear vein at a rate of 0.5 mL/min, in order to determine the pharmacokinetic parameters in rabbit. The blood samples were taken at 0.02, 0.5, 1, 2, 3.5, 4.5 and 6 h after injection. The transdermal administration of TZN was accomplished through the application of 13 cm^2 matrix-type transdermal device containing 50 mg of TZN to the inner pinna skin of the rabbits (group III) for a 24 h period. The blood samples were taken at 0, 2, 4, 6, 7 and 24 h. The group IV rabbits received the placebo transdermal formulation to the pinna skin, which acted as a control. The blood samples were taken at regular interval for 24 h.

Analysis of TZN

The blood sample (0.5 mL) was collected in centrifuged tube containing 0.2 mL of heparin as anticoagulant. The blood was extracted for TZN using microscale protein precipitation technique (11) with slight modification. To 0.5 mL of blood sample, 0.5 mL of 0.5 M NaOH and 0.5 mL of 10% $ZnSO_4$ in water were added. The mixture was centrifuged at 2798 × g for 15 min. The clear supernatant was filtered through 0.2 µm membrane filter and stored frozen until analysis.

The extraction method was validated for accuracy and precision by incorporating a known amount of TZN into 0.5 mL of normal saline and/or drug-free blood at two different concentrations and extracting the sample. The efficiency of this extraction was determined to be more than 90%.

TZN concentrations in the extracted samples were determined by HPLC. A Shimadzu HPLC system was used for the analysis. The system was equipped with a model series LC-AT-VP pump and a model series SPD-10A-VP ultraviolet detector. Separation and quantization were made on a 250 × 4.6 mm (i.d.) Phenomenex C-18 column (5 µm particle size). The mobile phase was prepared by mixing acetonitrile and phosphate buffer at pH 4.5 in a ratio of 30 : 70 v/v. The mobile phase was filtered through 0.2 µm membrane filter and degassed by sonication prior to use. The samples were also filtered through 0.45 µm disposable membrane filters. 20 µL of sample was injected onto the column at a flow rate of 1 mL/min. TZN concentrations were determined using an ultraviolet detector, set at a wavelength of 246 nm. All determinations were performed at ambient temperature. The calculations of TZN were performed using external standards and peak areas (12, 13). The standard curve was generated using standards with TZN concentrations of 0.01 – 4 mg/mL in control plasma. 20 µL injection was made for each concentration and chromatographed under the specified chromatographic conditions. The peak area values were plotted against concentrations. A linear standard curve was obtained over the concentration ranges investigated (r^2 = 0.9354). The linearity of the calibration curve was proved by the high value of coefficient of correlation. Data acquisition was performed using CSW 32 software (Shimadzu).

Under the described chromatographic conditions, sharp peak was obtained for TZN. The average retention time was found to be 7.94 ± 0.006 min. The value obtained in our study agrees with the reported values (1, 11). The TZN was clearly resolved from endogenous interferences and there were no interfering peaks in the control plasma sample.

Data treatment

The results are expressed as the mean values ± SEM. The cumulative amounts of TZN released and permeated per unit diffusion surface area (Q, µg/cm^2) were plotted against time (t, h), and the slope of the linear portion of the plot was estimated as steady-state flux (J_{ss}, µg/cm^2/h). The lag time, t_L (h), was determined from the x-intercept of the slope at the steady state. The pharmacokinetics of TZN was analyzed for the rabbit after the data were fit to one-compartment open model with the appropriate route of administration. The parameters such as maximum concentration of drug in plasma, (C_{max}, µg/mL) and the time for the drug to reach maximum concentration in the plasma after drug administration, (T_{max}, h), were computed directly from the measured plasma concentration data. The steady-state drug concentration (Css, µg/mL) in plasma after transdermal administration was considered achieved when there was no significant difference between drug concentrations of consecutive sampling times. The elimination rate constant (β, h^{-1}) was estimated from the terminal slope of the individual plasma concentration-time curve after logarithmic transformation of plasma concentration values. The elimination half-life ($t_{1/2}$, h) was calculated from the relation 0.693/β. The area under the plasma drug concentration-time curve, $AUC_{0\text{-}Ct}$ was calculated using the linear trapezoidal rule and the latter by dividing the last measurable plasma drug concentration with elimination rate constant to deter-

mine $AUC_{Ct-\infty}$. The sum of these two areas was the estimate of total area under the curve ($AUC_{0-\infty}$). The clearance of TZN was calculated by multiplying the volume of distribution by its elimination rate constant.

Statistical comparisons were made using Student's t-test. The chosen level of significance was $p < 0.05$.

RESULTS AND DISCUSSION

Ex vivo skin permeation of TZN from the matrix patch across mouse epidermis demonstrated a linear Q versus t relationship ($Q = 134.09\ t - 512.41$, $R^2 = 0.9848$) after an initial lag time of 3.5 h. This relationship indicates that TZN permeates through the intact mouse skin at a constant rate. The *ex vivo* skin permeation profile of TZN following application of the matrix device to the mouse skin is shown in Figure 1. A steady state skin permeation rate of 134.09 ± 2.49 μg/cm^2/h was achieved after an initial lag time of 3.5 ± 1.0 h.

Figure 2 represents the plasma concentration-time profile after the intravenous injection of TZN. The data were fit to the one-compartment open model and the different pharmacokinetic parameters were estimated. A summary of the pharmacokinetic parameters of TZN after intravenous injection is shown in Table 1.

The results from the oral administration of TZN solution indicate that TZN is rapidly absorbed from the rabbit GI tract with a C_{max} of 5.84 μg/mL at a T_{max} of 2 h (Figure 3). No significant difference ($p < 0.05$, Student's *t*-test) in the elimination half-life of TZN was observed after intravenous (2.7 h) and oral administration (2.4 h). The elimination rate constant (β, h^{-1}) after intravenous and oral administration of TZN was 0.4 h^{-1} and 0.3 h^{-1}, respectively.

Transdermal administration of TZN via the matrix type device achieved C_{max} of TZN to 3.1 μg/mL at a T_{max} of 5 h after an initial lag time of 2 h (Figure 4). The steady-state concentration was calculated to be 2.3 μg/mL (7 h – 24 h) (Figure 4). It was noted that the attained C_{max} in oral treatment persisted for a short duration and then the plasma drug concentration continued to decline while in the case of transdermal treatment it was almost maintained for 24 h (period of study). The pharmacokinetic parameters for oral and transdermal administration are presented in Table 2.

The observed *in vivo* steady-state plasma concentration (2.3 μg/mL) after transdermal administration in rabbit is in good agreement with simulated steady-state concentration. The simulated concentration (2.5 μg/mL) was calculated at steady-state from the equation reported by Tenjarla et al. (14) using the *ex vivo* flux as the rate of input (134.09 ± 2.94 μg/cm^2/h) and the total body clearance of 26.45 mL/h/kg determined from the intravenous pharmacokinetic study. The *ex vivo* t_L value of 3.5 h does correlate with the *in vivo* t_L value of 2 h. However, the slight inconsistency in the *ex vivo* and *in vivo* t_L value may be from the presence of the

Table 1. Pharmacokinetic parameters of TZN following intravenous injection in rabbits[a].

Parameters	Mean ± SEM
β, h^{-1}	0.25 ± 0.05
β-half life, h	2.7 ± 0.48
AUC, μg/mL × h	189.01 ± 51.97
Volume of distribution, mL/kg	105.81 ± 34.45
Clearance, mL/kg/h	26.45 ± 9.86

[a] Dose of 5 mg/kg, n = 3.

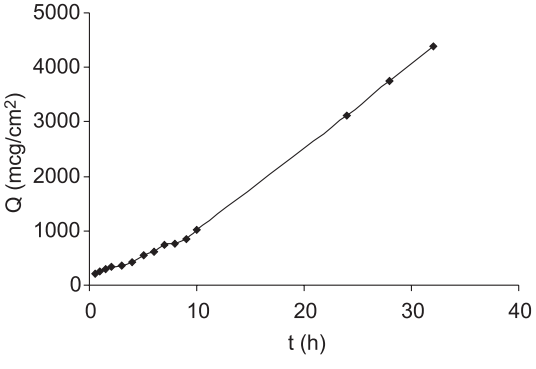

Figure 1. *Ex vivo* permeation profile of TZN following the application of the matrix patch to the mouse epidermis.

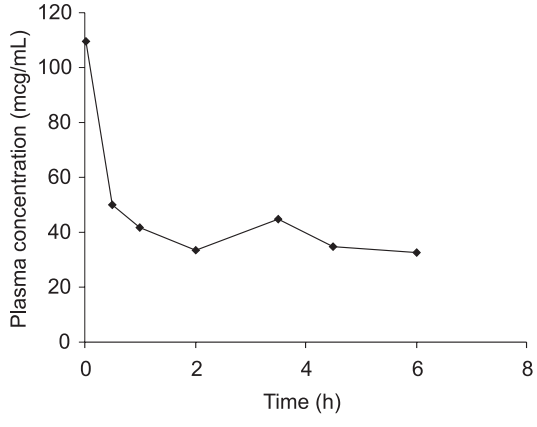

Figure 2. Plasma concentration versus time profile of TZN after intravenous administration (5 mg/kg) in rabbit.

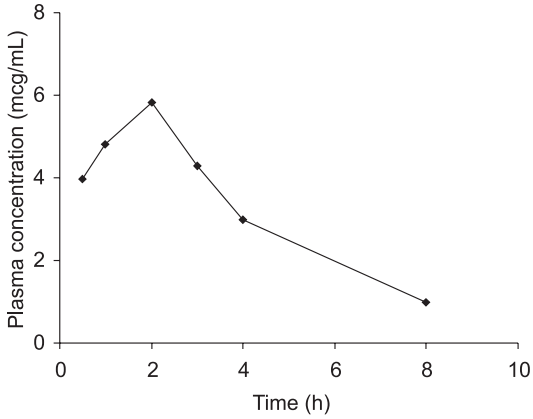

Figure 3. Plasma concentrations versus time profile of TZN following oral solution administration (25 mg/kg) in rabbit.

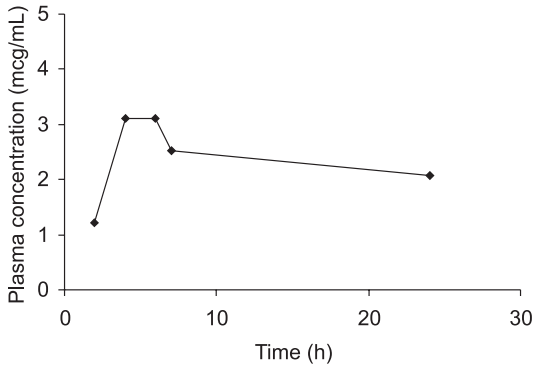

Figure 4. Plasma concentration versus time profile of TZN after application of matrix type transdermal device in rabbit.

Table 2. Pharmacokinetic parameters of TZN following oral and transdermal administration in rabbits[b].

Parameters	Transdermal	Oral
C_{max} (mg/mL)	3.1 ± 0.97	5.84 ± 2.31
Css (mg/mL)	2.3 ± 0.56	-
T_{max} (h)	5 ± 1.7	2 ± 0.55
AUC (μg/mL × h)	51.58 ± 8.63	28.66 ± 6.45
β, h^{-1}	-	0.29 ± 0.10
β-half life, h	-	2.4 ± 0.46

[b] Values are expressed as the mean ± SEM, n = 3.

microcirculation under the epidermis in intact living skin (15).

Ideally, the transdermal formulation should be evaluated in humans. The study in humans is restricted due to possible toxicities or adverse reactions of drugs and the insufficient availability of excised human skins directs the use of skins from laboratory animals like mice, rats and rabbits in the development and evaluation of transdermal formulations. Some studies indicate the barrier property similarity between the hairless animal skins (mice, rats) and the human (16, 17). But there are no substantive data to indicate their validity for the estimation of human skin permeability. On the other hand, there are reports that denote the sameness between humans and pigs for skin permeability of drugs (18, 19). The high degree of sameness in permeability between pig and human skin might be due to the similarity in skin surface lipids, barrier thickness and morphological aspects (19). The present study denote the likeness between mice and rabbits for skin permeability of TZN since there is a close parallelism in *ex vivo* (simulated) and *in vivo* steady state concentration and *ex vivo* and *in vivo* t_L. However, the permeability data from humans are yet to be substantiated for the skin from mice and rabbits.

CONCLUSIONS

The skin permeation of TZN following the application of matrix type TDDS of TZN occurred at a controlled rate indicating zero-order kinetics. The plasma level of TZN following transdermal application could be maintained for 24 hours. Compared with oral TZN solution administration, the transdermal preparation of TZN resulted in low peak plasma levels after an initial lag time. The T_{max} value was considerably high. The transdermal dose achieved a much higher steady-state blood concentration in rabbits (2.3 μg/mL) compared with the effective blood concentration in human (0.75 μg/mL) (1, 3, 11). The clearance of TZN in rabbit was estimated to be 26.45 mL/h/kg. Theoretically, with the observed *ex vivo* skin permeation rate and *in vivo* clearance value, a higher systemic blood con-

centration (2.5 μg/mL) is to be expected. However, the clearance value of TZN in human is 126 mL/h/kg (2, 20). Taking this clearance value into account, the 2.5 μg/mL steady state blood concentration may appears to be within the expected therapeutic range in human.

Acknowledgment

Authors are grateful to University Grants Commission, New Delhi for financial support.

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Received: 18.01.2008