The Prediction of Plasma and Brain Levels of 2,3,5,6-Tetramethylpyrazine Following Transdermal Application

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ABSTRACT

The purpose of this study was to construct a pharmacokinetic (PK) model and to determine PK parameters of 2,3,5,6-tetramethylpyrazine (TMP) after application of TMP transdermal delivery system. Data were obtained in Sprague-Dawley (SD) rats following a single dose of TMP transdermal delivery system. Blood samples were obtained at 0, 0.25, 0.5, 1, 2, 4, 6, 16, and 24 hours after the transdermal application. In the brain level study, 18 SD rats were divided into 6 groups. Three SD rats before and after transdermal application were culled and sacrificed at each of the following time intervals: 2, 4, 6, 16, and 24 hours after the TMP-TTS application. TMP concentrations in plasma and brain tissues were determined using high performance liquid chromatography and data were fitted using a zero-order absorption and a first-order-elimination 3-compartment PK model. Fitted parameters included 2 volumes of distribution (V_1 , V_2) and 2 elimination rate constants (k_{10} , k_{20}). The elimination half-life for TMP in plasma and brain was 26.5 and 31.2 minutes, respectively. The proposed PK model fit observed concentrations of TMP very well. This model is useful for predicting drug concentrations in plasma and brain and for assisting in the development of transdermal systems.

KEYWORDS: tetramethylpyrazine, percutaneous absorption, transdermal drug delivery system, in vitro/in vivo, pharmacokinetic model

INTRODUCTION

The biologically active ingredient 2,3,5,6-tetramethylpyrazine (TMP, structure shown in **Figure 1**), originally isolated from *Ligusticum wallichii* Franch in 1957, has been used routinely in China for the treatment of stroke and angina pectoris.^{1,2} It was recognized as a candidate for a cognitive enhancer, as it showed significant actions such as improving brain microcirculation and blood viscosity.³ It was reported recently that following an intravenous administration of 2, 5, or 10 mg/kg to rats, TMP penetrated through the blood-brain barrier within 10 minutes. The concentrations of TMP in various regions of the brain (cerebral cortex, brainstem, striatum, hippocampus, cerebellum, and midbrain) were not significant at 15 minutes.⁴ Analysis of the relationship between brain and blood levels yields a progressive brain/blood ratio suggesting appreciable blood-brain barrier penetrability of TMP.⁵

Oral administration of TMP is known to exhibit extensive first-pass metabolism, low oral bioavailability (10%-30%), and short biological half-life of 0.5 to 2 hours.^{6,7} TMP can be given orally 100 mg 3 times daily. To maintain therapeutic blood levels for long periods, intravenous infusion is needed in the treatment; for instance, TMP hydrochloride injection (40 mg/2 mL) or TMP phosphate injection (50 mg/2 mL) is diluted with 250 to 500 mL of isotonic saline or glucose solution and intravenously infused for 4 to 6 hours.⁸ For maintenance therapy, controlled release through an alternative route may be more meaningful, since frequent oral dosing and 4 to 6 hours of intravenous infusion may lead to non-compliance.

It is very interesting that *L. wallichii* Franch, the herbal medicine from which TMP originally was extracted, is a primary component in several Chinese medicated plasters for local and systematic pharmacological actions.^{9,10}

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Transdermal drug delivery offers many advantages, such as improvement of bioavailability by avoidance of first-pass metabolism, and elimination of side effects by reduction of plasma peak and trough ratio.¹¹ The clinical effectiveness of *L wallichii* Franch contained in Chinese medicated plasters encouraged us to investigate the potential percutaneous permeability of TMP. Although there are plenty of centuries-old records testifying to the clinical power of those plasters, little support from basic scientific data is available. To develop better and more effective drugs or drug delivery devices for Chinese medicines, we worked to integrate Chinese medical knowledge with Western medical technology.¹²

Figure 1. Structure of TMP.

Our studies on physicochemical properties and percutaneous penetration of TMP showed that TMP is a potential candidate for delivery across the skin.^{13,14} TMP has a molecular weight of 136.2. Its melting point is 76°C to 78°C, and its solubility in water is 11 mg/mL. The partition coefficient ($k_{\text{oct/water}}$) of TMP was found to be 123.5; the $\log k_{\text{oct/water}}$ was thus 2.3. An aqueous solution of TMP without any enhancer has a permeation rate of $\sim 90 \mu\text{g}/\text{cm}^2/\text{h}$ through human cadaver breast skin. To reach the therapeutic level, the transdermal patch needs to be about 40 cm^2 (Qi et al., unpublished data, 2002).^{15,16}

In this study, we proposed a pharmacokinetic (PK) model of transdermal TMP to enable the correlation of in vitro and in vivo data and described PK parameters. Franz-type diffusion cells were used for in vitro permeation rate determinations. In vivo experiments were performed with Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

TMP phosphate was purchased from Liming Pharmaceutical Company (Guangzhou, China). Carbamazepine (purity > 99.5%) was obtained from Shanghai Chemical Compound Company (Shanghai, China). SD rats (250–300 g) were obtained from the Animal Center of Shanghai Institute of Industrial Pharmaceutics (Shanghai, China). Modified Franz diffusion systems with a 7.0-mL

capacity of receiver compartment and a 2.54-cm^2 diffusion surface area were made by the Technical Support Department, Shanghai Institute of Industrial Pharmaceutics. TMP transdermal delivery system, prepared in our lab, is a reservoir-type transdermal system using ethylene vinyl acetate (EVA) copolymer membrane as a rate-controlled membrane.

Drug release kinetics studies

Drug release studies were conducted in Franz-type diffusion cells at 37°C. The patch was placed between the donor and receiver compartments. The receptor compartment contained 7.0 mL of water. The diffusion surface area was 2.54 cm^2 .

At suitable time intervals, the whole receiver phase was taken for drug assay. The release of drug from the TMP patch was tested for 24 hours and determined in 3 sets of diffusion cells. Assays of TMP were performed using a Shimadzu LC-10A liquid chromatography system equipped (Kyoto, Japan) with a SPD-10A UV-vis detector (Kyoto, Japan), and a Hewlett-Packard data module (Palo Alto, CA). A reversed-phase YMG-C18 column (20 cm x 4.6 mm, Changchun Applied Chemistry Research Institute, Chuangchun, China) was utilized as the analytical column and maintained at 35°C. The mobile phase consisted of 35% methanol and 65% water, adjusted to pH 5 with phosphoric acid. The flow rate was 1.0 mL/min. The injection volume was 10 μL . The detector wavelength was set at 280 nm. A linear peak area versus concentration relationship was established with a correlation coefficient >0.999.

In vitro percutaneous penetration kinetics

The skin samples were obtained from the abdominal regions of SD rats. Rats were sacrificed by cervical dislocation. The abdominal skins were removed by blunt dissection. The abdominal fur was removed using clippers. All skin samples, in full thickness, were cut and washed with water. Fat and connective tissues were carefully removed with a scalpel. Skin was examined for any damage through a magnifying lens. The skin specimens were wrapped in plastic film and stored in a freezer at -20°C until they were needed. The frozen skin samples were thawed to room temperature before mounting them within the diffusion apparatus.

The same Franz-type diffusion cell as described above was used to investigate in vitro skin penetration kinetics at 37°C. The skin pieces, placed on the receiver cell, were equilibrated with the receiver phase for 2 hours before beginning the experiment. Then the receiver phase was renewed and the TMP transdermal delivery system

were put in place. At regular intervals up to 24 hours, the whole receiver phase was taken for drug assay, as described above. Tests were performed in triplicate and averaged.

Animal studies

To reduce the risk and to minimize the cost of human study, rats were used as an animal model for bioavailability study of transdermal delivery systems. It should be noted that rat studies may not be precisely predictive of human skin permeation. Moreover, there is always the possibility that the skin might be damaged in the pretreatment and become more permeable.¹⁷⁻²⁰ The SD rats (250-300 g) were housed in stainless steel cages before treatment. One day prior to the experiments, their abdominal side hair had been carefully shaved. No visible signs of damage on the skin surface were observed. On the following day, the TMP patch was applied on the back skin of each rat for up to 24 hours. Blood samples were obtained at 0, 0.25, 0.5, 1, 2, 4, 6, 16, and 24 hours after the patch application. In the brain level study, 18 SD rats were divided into 6 groups of 3 rats each. Three rats before and after transdermal applications were killed at each of the following time intervals: 2, 4, 6, 16, and 24 hours. Brain tissue was removed and weighted, homogenized with saline (1:4) under a -20°C ice bath. Each single point on the drug/brain concentration profile corresponds to a group of 3 rats.

Analytical assay of plasma and brain samples

TMP concentration in plasma and brain was determined using a high performance liquid chromatography (HPLC) method. The lower detection limits were 5 ng/mL and 10 ng/g for plasma and brain samples, respectively. The HPLC systems consisted of a Shimadzu LC-10A pump, SPD-10A UV-vis detector, and Hewlett-Packard data module. The mobile phase was a mixture of acetonitrile, 0.02N sodium phosphate, and water (30:65:5). The flow rate was 1.0 mL/min. The UV detector was set at 280 nm. The column temperature was performed at 35°C. Carbamazepine was used as the internal standard. To each 200-μL blood sample or 100-ng brain sample, 20 μL of the internal standard (10 μg/mL) and 100 μL of 20% ammonium solution (pH = 8.2) were added. The mixture was shaken for 2 min and then extracted by methylene dichloride and then centrifuged at 3500 rpm for 10 min. 20 μL of the organic phase was withdrawn and added to 100 μL of a 10% hydrochloric acid methanol solution. Then the upper layer was dried at 40 ~ 50°C. The dried samples were stored at -80°C until analysis. Upon analysis, the samples were reconstituted with 200 μL of acetonitrile. 10 μL was used for HPLC analysis.

This bioanalytical method had a sensitivity limit of 5 ng/mL for blood samples and 2.5 ng/g for brain samples. The coefficient of variation was 0.8% to 1.4%. The extraction recoveries were >90% at all concentrations. The HPLC assay method was validated over a range of 0.050 to 100.0 μg/mL for blood samples or 0.025 to 50.0 ng/g for brain samples. The intra- and inter-day precision ranges were <5.0%.

PK modeling

Compartmental PK techniques were used to establish a theoretical profile of the plasma and brain concentrations after transdermal administration. PK models were designed and investigated based on visual inspection of graphs (plasma/brain concentration time course), inspection of weighted residuals (difference between fitted and observed concentrations) versus observed concentrations, and values of Akaike's information criterion test.²¹

The in vitro drug release testing (**Figure 2**) showed that the release kinetics from TMP-TTS are in a biphasic pattern characterized by a rapid release during the first 2 hours (burst release), followed by a slow release from 2 to 16 hours (release rate > 20 mg/h). The in vitro skin permeation pattern (**Figure 3**) showed that the in vitro skin permeation, k_0 , was 7.1 mg/h with 1.0 hour of lag time. Since the release rate from the patch is higher than the skin permeation rate, the percutaneous absorption of TMP from the transdermal delivery system is limited by the skin permeability (Qi et al, unpublished data, 2002).^{15,16} Therefore, the release rate from the transdermal delivery system was not included in the model.

The biological nature of the blood-brain barrier²² was taken into account in the model selection. Since the transport of drug between blood and brain could reach equilibrium, 2 rate constants, k_{12} (from blood compartment to brain compartment) and k_{21} (from brain compartment to blood compartment), were used. TMP may undergo metabolism in brain²³ or may be eliminated during inhibiting platelet formation,²⁴ so an elimination rate from brain compartment, k_{20} , was introduced in the model selection.

Therefore, fitted parameters in the PK model included (1) the first-order elimination rate constant from the central compartment, k_{10} and peripheral compartment, k_{20} ; and (2) the mass transfer constants between the central and peripheral compartments, k_{12} and k_{21} . This model is schematically illustrated in **Figure 4**. V and C represent the volume of distribution (V/F) and drug concentration, respectively, where F represents the bioavailability.

Figure 2. In vitro drug release profiles of TMP-TTS. Each point represents the average of 3 measurements.

Figure 3. Cumulative amount permeated versus time profile of TMP through rat skin. Each point represents the average of 3 measurements.

Figure 4. Schematic representation of the 3-compartment (skin, plasma, brain) model. The rate constants that apply to mass transfer between the different compartments are indicated by k_{12} and k_{21} .

The linear differential equations describing the mass transfer process between the different compartments can be written as follows

$$V_1 \frac{dC_1}{dt} = k_0 - (k_{10} + k_{12})V_1C_1 + k_{21}V_2C_2 \quad (1)$$

$$V_2 \frac{dC_2}{dt} = k_{12}V_1C_1 - (k_{21} + k_{20})V_2C_2 \quad (2)$$

Initial condition: when $T = 0$,

$$C_1(0) = 0, C_2(0) = 0 \quad (3)$$

Prior to the model fitting, the values of k_0 were used from the in vitro skin permeation data. The values of V_1 , V_2 , k_{10} , k_{20} , k_{12} , and k_{21} were obtained following the model fitting by using the WinNonlin program.²⁵ The elimination half-life for TMP in plasma (half-life, 1) and brain (half-life, 2) was also determined as the secondary parameter. The correlation coefficients between Y and Y predicted (R^2), Akaike Information criteria, indexed together with the sum of weighted squared residuals, were examined to determine the goodness of fit.²⁵

RESULTS AND DISCUSSION

PK modeling of transdermal drug delivery is a valid approach to predict skin penetration and to examine and optimize a formulation strategy.^{26,27} In this study, we propose a PK model of transdermal TMP to enable the correlation of in vitro and in vivo data and described PK parameters. The proposed PK model provided a very good fit to the observed drug plasma data and drug brain data following transdermal application of TMP, as shown in **Figure 5**.

The fit was very good, as demonstrated by the high value of correlation coefficient ($R^2 = 0.98$) and low value of weighted residuals and Akaike's index (data not shown). The visual examination of graphs of the weighted residuals versus observed plasma/brain concentrations showed no systemic bias or deviation. Predicted PK parameters are summarized in **Table 1**.

The present results suggest that the pharmacokinetics of TMP appear to best fit the kinetics of a 3-compartment model in skin, central blood, and central nervous systems. Analysis of the relationship between brain and blood levels suggests appreciable blood-brain barrier penetrability, which supports the claim for central nervous system activities for TMP following transdermal administration. Such an observation is compatible with expectations for an agent possessing central nervous system activities.²⁸

Assuming equilibrium was reached between the 2 compartments, equation 2 could be rewritten:

$$V_2 \frac{dC_2}{dt} = k_{12}V_1C_1 - (k_{21} + k_{20})V_2C_2 = 0 \quad (4)$$

Hence,

$$C_1/C_2 = (k_{21}V_2 + k_{20}V_2)/(k_{12}V_1) \quad (5)$$

As shown in **Table 1**, the predicted values for V_1 , V_2 , k_{12} , k_{21} , and k_{20} were 458.96 mL/kg, 65.75 mL/kg, 2.22 h^{-1} , 20.14 h^{-1} , and 1.33 h^{-1} , respectively. Therefore, the ratio of drug levels in brain and blood at equilibrium stage, C_1/C_2 , was calculated from Equation 5 and found to be 1.38. The experimental values of the ratio of drug levels in plasma and brain at 4 hours and 6 hours were 1.34 and 1.45, respectively. The predicted value of TMP plasma-brain level ratio was close to the experimental

Figure 5. Observed TMP plasma concentration (o), fitted TMP plasma level (—), observed brain concentration (•), and fitted brain level (----) versus time after an application of TMP-TTS.

Table 1. Predicted Tetramethylpyrazine Pharmacokinetic Parameter Values After Transdermal Application

Pharmacokinetic Parameters	Value
V_1	458.96 (mL/kg)
V_2	65.75 (mL/kg)
k_{12}	2.22 (h^{-1})
k_{10}	1.57 (h^{-1})
k_{21}	20.14 (h^{-1})
k_{20}	1.33 (h^{-1})
Half-life, 1	26.5 (min)
Half-life, 2	31.2 (min)

observation, suggesting that equilibrium was reached at 4 to 6 hours between the brain and blood compartment.

Calculated PK parameters of TMP presented are also consistent with prior reports.^{4,5} The terminal elimination half-lives are independent of the mode of administration. The mean TMP plasma elimination half-life of 26.5 minutes in this study is similar to values published following the intravenous administration. Moreover, the

predicted TMP brain elimination half-life of 31.2 minutes was also close to the reported value for intravenous and oral delivery.^{4,5} This observation leads to the hypothesis that there is probably no reservoir effect of the drug in the application site from the absorption process, which is also consistent with the in vitro skin permeation findings. We noted that 1 hour after stopping the 8-hour in vitro skin permeation study, no detectable TMP was found in the receiver phase.¹⁶

Overall, we concluded that the PK model presented in this study allowed us to predict plasma and brain levels of TMP following transdermal application and was a simple and useful approach to improving formulation strategies for this type of system. By using this simulation, one can also predict TMP concentration in plasma and brain, and obtain a specific plasma level. The approach requires knowledge of in vitro release properties of the device as well as predetermined drug PK data. This work also showed that TMP-TTS could provide a well-controlled plasma and brain level. TMP transdermal delivery system possesses advantages such as avoidance of first-pass metabolism, improvement of patient compliance, and reduction of bioavailability problems in comparison with other administration routes.

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