

***In vitro* permeation of tetramethylpyrazine across porcine buccal mucosa**

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ABSTRACT

AIM: To examine the *in-vitro* transport route of tetramethylpyrazine (TMP) across porcine buccal mucosa and to investigate the effects of drug concentration, pH in donor chamber, and 1-octanol/buffer partition coefficient on transbuccal permeation.

METHODS: *In-vitro* permeation of TMP through porcine buccal mucosa was studied by using in-line flow through diffusion cells at 37 °C. The permeability of TMP was evaluated at different donor pH and drug concentration. Permeability of unionized (P_u) and ionized TMP (P_i) was calculated by using the Scientist[®] software. **RESULTS:** The steady state flux of TMP increased linearly with the donor concentration ($r^2=0.96$) at pH 7.4. The permeability and the partition coefficient increased with pH. P_u and P_i were $9.05 \times 10^{-6} \text{ cm}\cdot\text{s}^{-1}$ and $2.99 \times 10^{-7} \text{ cm}\cdot\text{s}^{-1}$, respectively. The total permeability coefficient increased with the fraction of unionized form. **CONCLUSION:** TMP permeated through buccal mucosa by a passive diffusion process. The partition coefficient and pH dependency of drug permeability indicated that the drug was transported mainly via the transcellular route by a partition mechanism.

INTRODUCTION

The examination of penetration route for transbuccal drug delivery is important because it is fundamental to select the proper penetration enhancer to improve the drug permeability. Based on the cellular structure of the oral mucosa, there are two possible pathways for passive drug transportation - the paracellular route and the transcellular route. The physicochemical properties of the diffusant determine the dominant route. For

lipophilic compounds, the transcellular pathway is the main route. For hydrophilic compounds, paracellular transport is the primary route.

The flux of drug through the membrane under sink condition for paracellular route can be written as^[1]:

$$J_p = \frac{D_p \varepsilon}{h_p} C_d \quad (1)$$

D_p : diffusion coefficient of the permeate in the intercellular spaces

h_p : pathlength of the paracellular route

ε : area fraction of the paracellular route

C_d : donor drug concentration

The flux of drug through the membrane under sink condition for transcellular route can be written as^[1]:

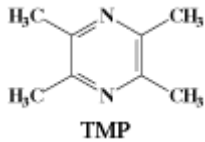
$$J_c = \frac{(1 - \varepsilon) D_c K_c}{h_c} C_d \quad (2)$$

K_c : partition coefficient between lipophilic cell membrane and the aqueous phase

D_c : diffusion coefficient of the drug in the transcellular spaces

h_c : pathlength of the transcellular route

Tetramethylpyrazine (TMP), isolated from the traditional medicinal herbs *Ligusticum wallichii* Franch. or *L. chuanxiong* Hort, has been widely used in China for the treatment of cardiovascular and cerebrovascular disease^[2]. TMP is a basic drug with short half life ($t_{1/2} = 2.89$ h)^[3]. Frequent administration of this drug is needed to keep an effective plasma concentration. Drugs delivered through mucosa can enter systemic circulation and bypass the hepatic first-pass effect. The non-keratinized buccal mucosa offers higher permeability compared to the transdermal route. Therefore, delivery of TMP for systemic use *via* buccal mucosa is investigated. The present study is to examine the effects of drug concentration at pH 7.4, pH in donor chamber, and 1-octanol/buffer partition coefficient on transbuccal permeation and the possible *in-vitro* transport route of tetramethylpyrazine (TMP) across porcine buccal mucosa.



MATERIALS AND METHODS

Chemicals and reagents Tetramethylpyrazine (lot: 990108) was purchased from Beijing Institute of Pharmaceutical Industry (Beijing, China). Citric acid monohydrate was purchased from J T Baker Chemical Co (Phillipsburg, NJ, USA). Sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of AR grade.

Collection and preparation of buccal tissue Porcine buccal tissue was selected as animal model for its high similarities to that of human being in term of structure and composition^[4]. Buccal tissue was obtained from a local slaughterhouse (Long Ranch Inc, Manteca, CA, USA) and stored in normal saline solution at 4°C upon collection. The mucosal membrane was separated by removing the underlying connective tissue with surgical scissors and mounted between donor and receiver chambers of the diffusion cells for permeation studies.

Analytical methods TMP was quantified by using HPLC. The HPLC system consisted of an SPHERI-5 RP-18 BrownleeTM column (Applied Biosystems), a Waters M590 pump, a Gilson 115-UV absorbance detector, and a Hitachi L7200 autosampler. Data were analyzed by EZChrome software (Scientific Software, CA, USA). The mobile phase was composed of methanol and distilled water at the ratio of 60:40. Flow rate is 1 mL·min⁻¹. TMP in sample was detected at 285 nm.

Transbuccal permeation studies^[5] *In-vitro* permeation of TMP through porcine buccal mucosa was studied by using in-line flow through diffusion cells (PermeGear, Riegelsville, PA) at 37 °C for 24 h. Temperature was maintained at 37 °C by water jacket surrounding the chambers. The receiver chambers were filled with McIlvaine buffer solution at pH 7.4 and the donor chambers were filled with TMP solution of different concentration at pH 7.4 or TMP saturated McIlvaine buffers of different pH (from 2.4 to 7.4). The receiver chambers were stirred with Teflon coated magnetic stirring bars. The samples were collected every 90 min by using a fraction collector (Gilson FC 205 Fraction collector, Middleton, WI, USA) and analyzed by HPLC. Experiments were conducted in triplicates for each condition. The total amount of drug penetrated through the membrane was determined and plotted as a function of time. The permeability coefficients (P) were calculated from the linear part of the curves as follows

$$P = \frac{dQ/dt}{AC_d} \quad (3)$$

A: the surface area of diffusion, cm²

dQ/dt : amount of TMP permeated per unit time at steady state, $\text{mg}\cdot\text{s}^{-1}$

C_d : donor drug concentration, $\text{g}\cdot\text{L}^{-1}$.

The permeability of TMP was evaluated at its saturated concentration of different pH (from 2.4 to 7.4). The steady state flux ($J_{ss} = P C_d$) of TMP at pH 7.4 was calculated at different drug concentration. Permeability of unionized (P_u) and ionized TMP (P_i) was calculated by using the Scientist[®] software (MicroMath Scientific Software, Salt Lake City, Utah, USA).

Solubility of TMP (C_s) determination at different pH The solubility of TMP at different buffer solutions was determined by shake-flask method. Extra drugs were put in the screw-capped glass scintillation vials with buffer solution and equilibrated under constant shaking at $37\pm 0.5^\circ\text{C}$ for 24 h. The aqueous phase was then separated and diluted. The concentration of TMP in this phase was determined by HPLC.

Partition coefficient determination at different pH 1-Octanol was used to represent the biomembrane. The partition coefficients between 1-octanol and McIlvaine buffer solutions at different pH (from 2.4 to 7.4) were determined by shake-flask method. The two phases were mutually saturated before use. Equal volume of TMP buffer solution ($400\text{ mg}\cdot\text{L}^{-1}$) and 1-octanol were mixed in the screw-capped glass scintillation vials and equilibrated under constant shaking at $37\pm 0.5^\circ\text{C}$ for 24 h. The aqueous phase was then separated and the concentration of TMP in this phase was determined by HPLC. The partition coefficients ($K_{o/w}$) were calculated using the follow equation

$$K_{O/W} = \frac{C_{aqi} / C_{aqe}}{C_{aqe}} \quad (4)$$

C_{aqi} : initial concentration of TMP in buffer solution, $\text{g}\cdot\text{L}^{-1}$

C_{aqe} : equilibrium concentration of TMP in buffer solution, $\text{g}\cdot\text{L}^{-1}$

RESULTS

Effect of donor drug concentration on TMP flux at pH 7.4 The steady state flux of TMP at pH 7.4 increased with the donor concentration. A linear relationship was observed between the flux and drug concentration ($r^2=0.96$, Fig 1).

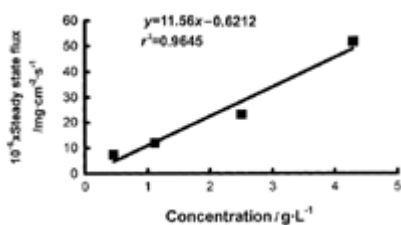


Fig 1. Effect of donor drug concentration on TMP steady state flux at pH 7.4.

Effect of pH in donor chamber The solubility of TMP at different pH buffer solution decreased with pH. The results were shown in Tab 1.

Tab 1. TMP solubility at different pH.

pH	3.0	4.0	5.0	6.8	7.4	8.0
Solubility/g·L ⁻¹	12.67	7.07	5.60	4.74	4.41	4.29

Both the apparent 1-octanol/buffer solution partition coefficient and the permeability coefficient of the drug increased with the pH of the buffer solution in donor chamber (Fig 2). Excellent linearity was observed between the permeability coefficient and the partition coefficient (Fig 3).

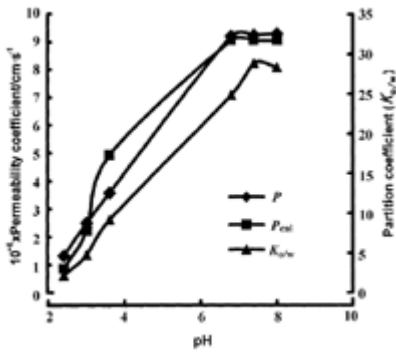


Fig 2. Effect of pH on permeability coefficient and partition coefficient of TMP.

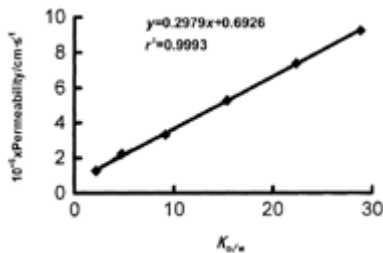


Fig 3. Correlation of 1-octanol/buffer solution partition coefficient and TMP permeability.

DISCUSSION

The linear relationship between the steady state flux at pH 7.4 and donor drug concentration showed that the transport of TMP through buccal mucosa at the concentration range from 0.46 to 4.29 g·L⁻¹ was a passive diffusion process (Fig 1).

According to equations mentioned above and the assumption that drug will have the same partition tendency at 1-octanol and the biomembrane, the permeability of drug should

have nothing to do with the partition coefficient if it goes through the paracellular route (Eq 1), while the permeability shall vary with the partition coefficient if the drug was transported via the transcellular route (Eq 2). Therefore, the transport route of drug permeation can be reflected since partition coefficient is pH dependent. In our study, the permeability of TMP was proportional to the partition coefficient (Fig 3) and both of them increased with pH (Fig 2), which showed a transcellular route to be the main pathway. To further explain our experiment results, we assume that unionized form mainly goes through transcellular route and the ionized form transports via the paracellular route, the steady state flux can be expressed by the following equation:

$$J_t = P_t C_t = J_u + J_i = P_u C_u + P_i C_i \quad (5)$$

J_t : total flux of TMP

J_u : transcellular flux

J_i : paracellular flux

C_u : concentration of unionized TMP

C_i : concentration of ionized TMP

C_t : total drug concentration

P_t : total drug permeability

so

$$P_t = P_u \frac{C_u}{C_t} + P_i \frac{C_i}{C_t} \quad (6)$$

TMP is a basic drug with pK_a of 3.51^[5]. The percentage of different species at a given pH can be calculated by using the Henderson-Hasselbalch equation.

$$pH = pK_a + \log \frac{[unionized]}{[ionized]} \quad (7)$$

P_u and P_i were calculated by fitting P_t , C_u/C_t , and C_i/C_t at different pH to equation (6). The calculated curve (P_{cal}) was plotted with the experimental data in Fig 2. The total permeability coefficient increased with the fraction of unionized form (Fig 4). The calculated value for P_u was $9.05 \times 10^{-6} \text{ cm} \cdot \text{s}^{-1}$, which is about 30 times higher than P_i , $2.99 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1}$. These results suggested that TMP transports via transcellular route based on our assumption. A limitation of our study was that the partition coefficient was determined from 1-octanol/buffer not biomembrane/buffer solution. Further studies such

as direct examination of the drug penetration will be conveyed to confirm the pathway of TMP.

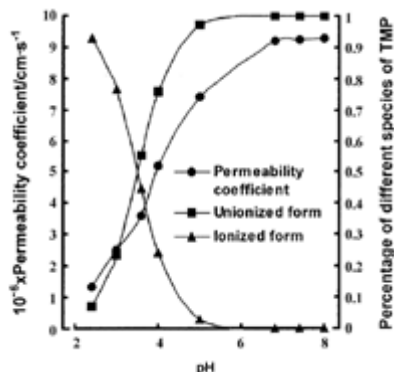


Fig 4. Relationship between permeability coefficient and the fraction of different species of TMP.

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