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## Synergistic effect of 1,4-cyclohexanediol and 1,2-hexanediol on percutaneous absorption and penetration of metronidazole

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## ABSTRACT

The objective of this study was to investigate the percutaneous absorption of metronidazole (MTZ) in the topical formulations containing a combination of 1,4-cyclohexanediol and 1,2-hexanediol. Six formulations were studied in an *in vitro* hairless mouse skin model using Franz Diffusion Cell. MTZ was applied at infinite doses (50 mg and 100 mg of the formulations, which correspond to 375 and 750 µg of MTZ, respectively). Based on the flux values and retardation ratio (RR), a synergistic retardation effect on percutaneous absorption of MTZ was observed for the formulations containing a combination of 1,4-cyclohexanediol and 1,2-hexanediol (RRs are 0.40 for 375 µg dose and 0.69 for 750 µg dose, respectively). Interestingly, retention of MTZ in epidermis and dermis layer showed no significant differences ( $p > 0.05$ ) between the formulations containing the retardant combination and control formulations. In other words, the retardant combination in the formulation decreases MTZ fluxes while maintaining similar level of retention in epidermis and dermis layer when compared to the control formulations. These observations provide insight in formulating superior topical formulations with minimized potential systematic toxicity while maintaining therapeutic efficiency. A mechanistic explanation of the observed synergistic effect is proposed.

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## 1. Introduction

Traditionally, topical formulations are designed to achieve penetration of active ingredient across the outermost layer of skin, the stratum corneum (SC), at therapeutically effective concentrations. The barrier property of the SC layer poses a formidable challenge to formulators of drug delivery systems (Cross and Roberts, 2000; Asbill and Michniak, 2000). Once the active ingredient penetrates across the SC layer, it could permeate through epidermis and dermis layer into systematic circulation, which might lead to unwanted or toxic side effects. For treatment of dermatological conditions, an ideal topical formulation is to impart maximal local retention and minimal systematic penetration. Furthermore, for agrochemicals (Baker et al., 1978), insect repellants (Briassoulis et al., 2001), sunscreens (Schlumpf et al., 2001), and household cleaning chemicals (Asbill et al., 2000; Mancini, 2004), minimizing potential toxicity is as important as providing protection benefits. As a result, there is a significant need in discovering safe and effective skin penetration retardants.

Recently, a number of studies have been carried out to prevent the passage of active ingredients or excipients into deeper skin layer by using chemical penetration retardants (Asbill and Michniak, 2000). The retardants could decrease the diffusivity and thermodynamic activity of the active ingredient in skin. Moreover, they will reduce amount of an active ingredient being released into the systematic circulation. Ideally, penetration retardants should be chemically and pharmacologically inert, nontoxic, non-irritant, and non-allergenic. They should have a rapid and reversible onset of action, be potent in low concentrations, compatible with the formulation ingredients and cosmetically acceptable (Chattaraj and Walker, 1995).

There are a limited number of publications in the area of penetration retardants. The retardants reported in the literature are usually structural analogues of potent enhancers. For example, Hadgraft et al. (1996) have reported that compounds having structure similar to Azone act as penetration retardants. It has been reported that a family of iminosulfurane compounds such as for example, *S,S*-dimethyl-*N*-(benzenesulfonyl) iminosulfurane, *S,S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane, *S,S*-dimethyl-*N*-(4-chlorobenzenesulfonyl) iminosulfurane has exhibited penetration retardation properties (Kim et al., 1999). In yet another example, oxazolidinones have been shown to be able to enhance retention of the applied active ingredients in the skin layer, resulting in low systematic permeation (Rajadhyaksha and Pfister, 1996; Seth, 1999). More recently, Li

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et al. (2010) reported that 1,4-cyclohexanediol in combination with 1,2-hexanediol showed a penetration retardation effect in percutaneous absorption of azelaic acid.

The present study was designed to investigate a potential synergistic retardation effect of 1,4-cyclohexanediol and 1,2-hexanediol on percutaneous absorption and penetration of an active agent. Furthermore, the study was also carried out to shine a light on mechanistic aspect of the retardation effect. metronidazole (MTZ) was selected as a model drug. MTZ is a member of nitroimidazole family of compounds. It is an active ingredient in a number of prescription pharmaceuticals for treatment of a number of conditions, such as, for example, rosacea. Percutaneous absorption and penetration of MTZ was studied in an *in vitro* skin model using Franz Diffusion Cell.

## 2. Materials and methods

### 2.1. Materials

MTZ was purchased from ALFA AESAR (ZhongAn pharmaceutical, Tianjin, China). 1,4-Cyclohexanediol was obtained from Sigma–Aldrich (St. Louis, MO, USA). 1,2-Hexanediol was purchased from Sabina Corporation (Piscataway, NJ, USA). Klucel® MF was obtained from Hercules, Inc. (Wilmington, DE, USA). All other chemicals are of analytical grade.

### 2.2. Skin membranes

Male hairless mice (30–40 days old) were purchased from Radiation Medicine Institute for Laboratory Animal Research, Chinese Academy of Medical Sciences (Tianjin, China). Animals were euthanized humanely. The abdominal skin was removed from hairless mice, and subcutaneous fat was carefully cleaned. All animal protocols were performed under the guidelines for humane and responsible use of animals in research set by Tianjin University School of Pharmaceutical Science and Technology. The skin samples were stored at  $-20^{\circ}\text{C}$  and were used promptly. For the formulation comparison studies, we have tried to make experimental conditions (including tissue conditions) as comparable as possible for all parallel experiments and taken all precautions to make a fair comparison. Before each experiment, the skin samples were thawed to room temperature and equilibrated at  $37^{\circ}\text{C}$  for 1 h in phosphate buffered saline (PBS, pH 7.4) in Franz Diffusion Cell.

## 3. Methods

### 3.1. Preparation of formulations

Six formulations were prepared (see Table 1 for details). Klucel® MF was used as the gelling agent. The general procedure is as follows. For example, to prepare formulation F4, 1% of 1,4-cyclohexanediol was dissolved in a solution of 1,2-hexanediol (4%) in water, MTZ (0.75%) was dispersed in the above solution with a stirrer until MTZ was dissolved. Then Klucel® MF (0.75%) was added to the solution while stirring until the solution was gelled. MTZ was completely solubilized in all formulations. The formulations have similar viscosity, which is about 50 cp.

### 3.2. *In vitro* skin permeation studies

The skin samples were mounted on Franz Diffusion Cell (Pharmacopoeia Standard Instrument Factory, Tianjin, China) with SC side facing the donor chamber (diffusion area =  $1.77\text{ cm}^2$ ). The receptor chamber (volume = 17 ml) was filled with PBS (pH 7.4), which is continuously stirred at 500 r.p.m. using a magnetic stir-

rer. The speed (500 r.p.m.) was optimized to maintain efficient mixing without creating air bubbles and vortex effect. The temperature was maintained at  $37 \pm 0.1^{\circ}\text{C}$ . Infinite doses were applied to the skin samples (50 mg and 100 mg of the formulations, which correspond to 375 and 750  $\mu\text{g}$  of MTZ, respectively). The donor chamber was sealed with Parafilm® to minimize evaporation of the formulations. Each set of experiments was run in six replicates. At the end of each time interval (1, 2, 4, 8, 12, 16, 20, and 24 h), the skin surface was wiped with cotton ball soaked with PBS (pH 7.4). The tape-stripping method (average 10 strips) was used to remove the SC layer (Howes et al., 1996). MTZ retained in the epidermis and dermis layer was collected by methanol extraction. After tape-stripping, the remaining skin was minced, vortexed with 1 ml methanol and centrifuged, the supernatant was removed. The extraction step was repeated three times. The supernatants were combined, filtered and ready for analysis.

### 3.3. HPLC analytical method

Analysis of MTZ was performed using HPLC (HP 1100, Agilent Technologies, Inc.) equipped with a 250 mm  $\times$  4.6 mm stainless steel  $\text{C}_{18}$  column (5  $\mu\text{m}$ , Thermo, USA). The mobile phase is a degassed and filtered (0.45  $\mu\text{m}$ ; Millipore) mixture of double distilled water–methanol (80:20, v/v). Injection volumes were 20  $\mu\text{l}$  and flow-rate was set at 1.0 ml/min. The UV detector wavelength was set at 310 nm for detection of MTZ.

The analytical method was validated for linearity, precision and accuracy. The correlation coefficient of 0.9997 for linearity of plot was observed. Intraday variability was less than 0.2% and interday variability was also calculated to be less than 3.0%.

### 3.4. Data and statistical analysis

For *in vitro* percutaneous absorption studies, three parameters (mean flux, lag time, and cumulative amount after 24 h) were calculated. The flux values of MTZ permeated through the skin membranes into the receptor fluid were determined from slopes of plots of concentration in the receptor phase as a function of time and expressed as  $\mu\text{g}/\text{cm}^2/\text{h}$  using linear regression (Microsoft Excel) (Batheja et al., 2009). The degree of penetration retardation is defined as the retardant ratio, RR, which is calculated from the following equation (Goodman and Barry, 1988):

$$\text{RR} = \frac{\text{Flux for the formulation containing retardants}}{\text{Flux for control formulation}}$$

Paired two-tailed Student's *t*-test is performed to calculate the statistical significance. Values are given as mean  $\pm$  SD.

## 4. Results and discussion

### 4.1. Flux values of MTZ

Hairless mouse skin tends to be thinner and has few layers in the SC than human skin. Therefore, it is more permeable than human skin (Catz and Friend, 1990; Fang et al., 2003). However, it is quite suitable to use the mouse skin for studying the permeation retardation effect, precisely due to its lower permeation barrier. One of reasons we chose mouse skin model is that if we were able to observe the retardation effect in more permeable mouse skin, it would have been more likely that a similar effect would have been observed in the less permeable human skin. It is expected that the retardation effect would be more profound in human skin.

The permeation parameters for F1–F2 in the presence and absence of 1,4-cyclohexanediol are summarized in Table 2. It is found that the flux values (1.67  $\mu\text{g}/\text{cm}^2/\text{h}$  for F1 and 1.69  $\mu\text{g}/\text{cm}^2/\text{h}$  for F2 at lower dose, 3.62  $\mu\text{g}/\text{cm}^2/\text{h}$  for F1 and 3.47  $\mu\text{g}/\text{cm}^2/\text{h}$  for

**Table 1**  
Formulations containing metronidazole (F1–F6).

Formulation	Ingredient (%)				
	Metronidazole	Klucel® MF	1,2-Hexanediol	1,2-Propanediol	1,4-Cyclohexanediol
F1	0.75	0.75	–	–	–
F2	0.75	0.75	–	–	1.0
F3	0.75	0.75	4.0	–	–
F4	0.75	0.75	4.0	–	1.0
F5	0.75	0.75	–	4.0	–
F6	0.75	0.75	–	4.0	1.0

F2 at higher dose, respectively) and the accumulated amounts of MTZ over a period of 24 h ( $Q_{24}$ ) are similar for formulations F1 and F2. RR values are about 1.0 for both formulations. Based on these data, it is concluded that 1,4-cyclohexanediol alone did not act as a penetration retardant for MTZ.

On the other hand, it is interesting to notice that regardless the dosing level, the flux values for the formulations containing a combination of 1,2-hexanediol and 1,4-cyclohexanediol are significantly lower than those for the formulations without 1,4-cyclohexanediol (formulations F3–F4 in Table 3). The effect is more profound at the 375  $\mu\text{g}$  dose as compared with at the higher dose (RR=0.40 vs. RR=0.69, flux value =  $1.49 \pm 0.10$  for F3 and  $0.60 \pm 0.01$  for F4, respectively). In addition, the lag times for F3 (1.10 h at lower dose and 0.98 h at higher dose) are longer than for F4 (1.53 h at lower dose and 1.20 at higher dose), suggesting a slow permeation of MTZ across hairless mouse skin after application of F4. Significantly lower amounts of MTZ were collected in the receptor medium at all time points for F4 as compared with F3 ( $p < 0.01$ ) (Figs. 1 and 2).

These results indicate that a combination of 1,2-hexanediol and 1,4-cyclohexanediol in the formulation exert a synergistic retardation effect on percutaneous absorption of MTZ.

In order to understand the role of 1,2-hexanediol in the observed effect, 1,2-hexanediol was replaced with 1,2-propanediol (formulations F5–F6) (Table 4). It was found that no statistical difference in terms of lag times, flux and  $Q_{24}$  values was observed between F5 and F6 ( $p > 0.05$ ). The RRs values for both formulations (F5 and F6) were approximately 1.0 at both dosing levels. These results show that there is no retardation effect observed in these two formulations (F5–F6). The observations confirm that either 1,2-hexanediol or 1,4-cyclohexanediol alone does not exert the retardation effect. A combination of 1,2-hexanediol and 1,4-cyclohexanediol, acting synergistically, is responsible for the observed retardation effect.

#### 4.2. Epidermal retention of MTZ

Treatment of many dermatological disorders relies on the ability of active agents to effectively penetrate the SC layer from applied formulations and reach dermatologically viable epidermis and dermis layer (Cross and Roberts, 2000). Ideally, a penetration retardant could minimize potential systematic penetration while maintaining retention in dermatologically viable epidermis and dermis layer.

The retention of MTZ in the epidermis and dermis layer was determined. At the 750  $\mu\text{g}$  dosage, percentage of MTZ retained in the epidermis and dermis layer was about 20% for both formulations F3 and F4. At the lower dosage, although the retention of MTZ decreases over the 24 h period after administration (from 25% at 1 h to 10% at 24 h), there is no significant difference in the retention of MTZ in the epidermis and dermis layer between F3 and F4 ( $p > 0.05$ ). Formulation F4 has a lower flux value and accumulated amount in the collection medium at 24 h ( $Q_{24}$ ), while maintaining a steady absorption of MTZ in the epidermis and dermis layer when compared to formulation F3. This is an interesting observation that a combination of 1,4-cyclohexanediol and 1,2-hexanediol in the formulation decreases systematic penetration of MTZ without reducing its retention in the epidermis and dermis layer.

#### 4.3. Discussion of skin penetration retardation

It is expected that solubility and partitioning play a minimal role in the observed retardation effect. This is based on our earlier publication (Li et al., 2010) and present study. In these studies, the retardation effect was observed for two APIs (MTZ and azelaic acid) with very different structures and properties (both physical and chemical). Azelaic acid is a saturated alkyl dicarboxylic acid, whereas metronidazole has a nitroimidazole ring structure. And yet, the retardation effect was observed in both cases. Thus, it is reasonable to assume that the diol combinations play a much important role.

Several publications suggest that the H-bonding power of a penetration enhancer or retardant is one of major factors in determining its skin penetration behavior (Abraham et al., 1995; Potts and Guy, 1995; Roberts et al., 1996). The most powerful H-bonding lipid in the SC layer is ceramide 6 (Wertz, 1992), which has four secondary alcohol and one secondary amide groups. Thus, interactions among ceramide 6 molecules are believed to represent the major intermolecular binding among SC lipids (Hadgraft et al., 1996).

Penetration enhancers, such as Azone, could intercalate into skin lipids due to its long lipophilic hydrocarbon chain, loosen up intermolecular binding among SC lipids, and increase the skin permeability (Lewis and Hadgraft, 1990; Hoogstraate et al., 1991). On the other hand, N-0915, an Azone analogue, has an extra oxygen atom which could form additional H-bonding with adjacent ceramide head groups on the either side, raising the possibility of crosslinking to both. Hadgraft et al. (1996) postulates that one-

**Table 2**  
Skin permeation parameters of MTZ at various doses (F1–F2 as control).

Formulation	Dose ( $\mu\text{g}$ )	$T_{\text{lag}}$ (h)	Flux ( $\mu\text{g}/\text{cm}^2/\text{h}$ )	Amount in collection medium at 24 h ( $\mu\text{g}$ )	RR
F1	375	$0.84 \pm 0.30$	$1.67 \pm 0.21$	$328.55 \pm 13.88$	–
F2	375	$0.82 \pm 0.20$	$1.69 \pm 0.41$	$326.62 \pm 15.71$	1.01
F1	750	$0.78 \pm 0.06$	$3.62 \pm 0.14$	$618.76 \pm 34.11$	–
F2	750	$0.75 \pm 0.02$	$3.47 \pm 0.29$	$621.98 \pm 41.92$	0.96

Each value represented the mean  $\pm$  SD ( $n = 6$ ).

RR, retardant ratio. RR = flux for the formulation containing retardation/flux for control formulation.

**Table 3**  
Skin permeation parameters of MTZ at various doses (F3–F4).

Formulation	Dose ( $\mu\text{g}$ )	$T_{\text{lag}}$ (h)	Flux ( $\mu\text{g}/\text{cm}^2/\text{h}$ )	Amount in collection medium at 24 h ( $\mu\text{g}$ )	RR
F3	375	$1.10 \pm 0.33$	$1.49 \pm 0.10$	$332.40 \pm 13.30$	–
F4	375	$1.53 \pm 0.10$	$0.60 \pm 0.01^*$	$215.90 \pm 6.91^{**}$	0.40
F3	750	$0.98 \pm 0.06$	$3.82 \pm 0.25$	$574.30 \pm 12.19$	–
F4	750	$1.20 \pm 0.37$	$2.63 \pm 0.03^*$	$508.62 \pm 18.43^{**}$	0.69

Each value represented the mean  $\pm$  SD ( $n=6$ ).

RR, retardant ratio. RR = flux for the formulation containing retardation/flux for control formulation.

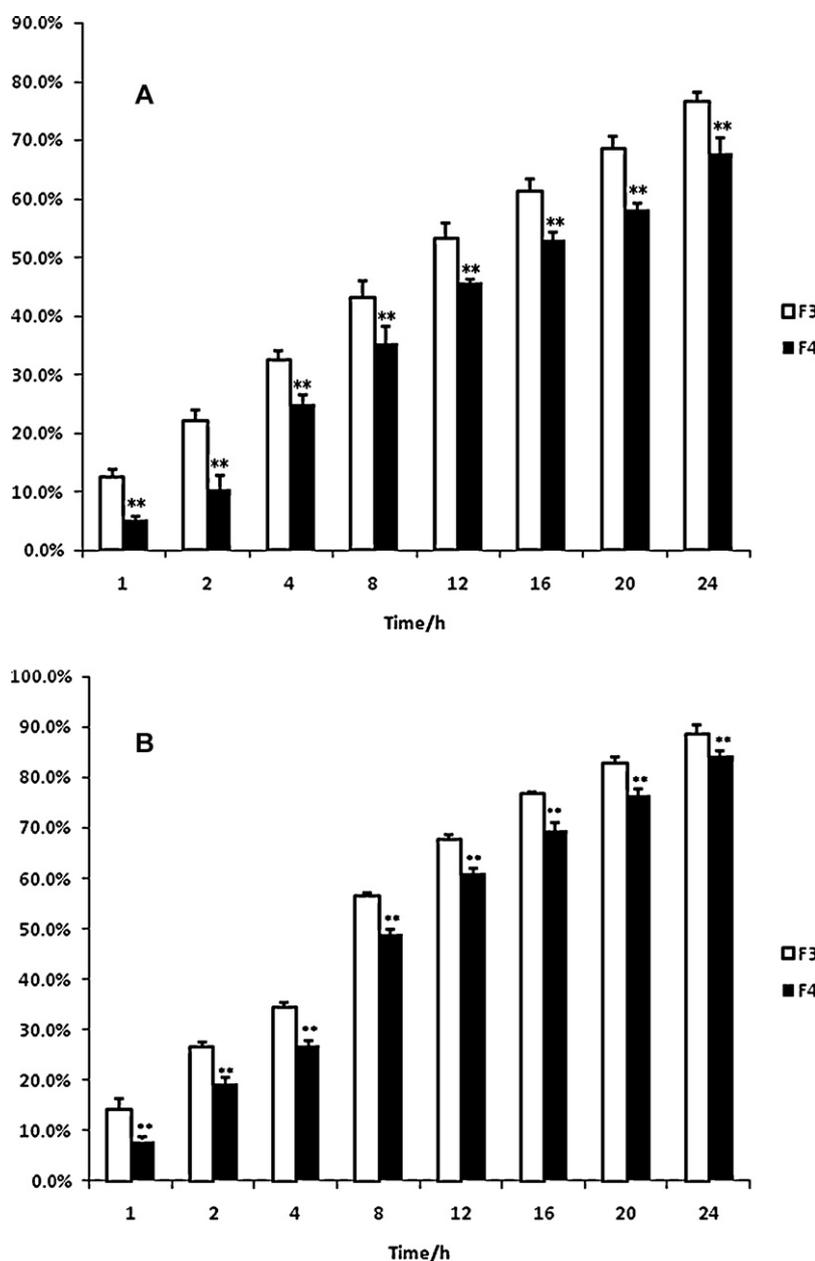
\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

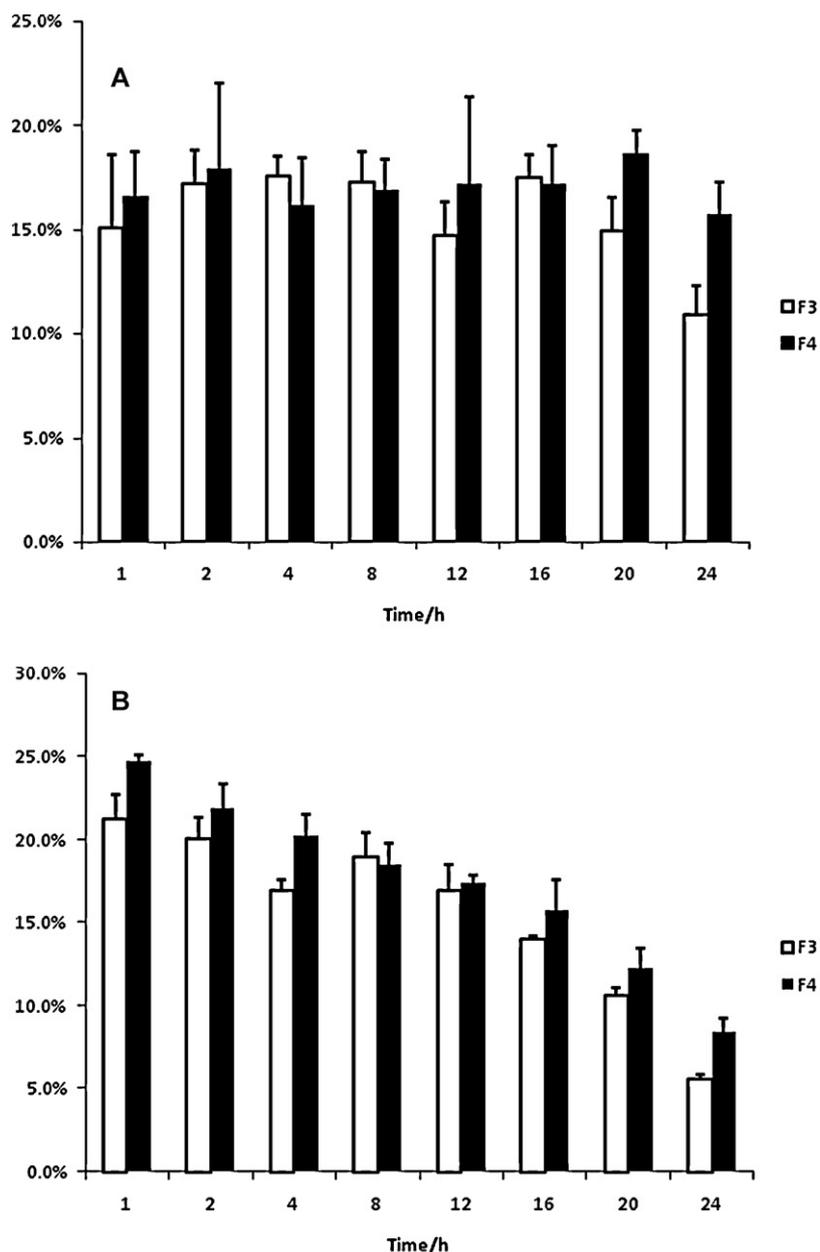
sided H-bonding between permeation modifiers and ceramide 6 promotes penetration enhancing activity, whereas two-sided interaction suggests a penetration retardation effect.

In present study, a combination of 1,2-hexanediol and 1,4-cyclohexanediol has shown a synergistic retardation effect on

percutaneous absorption of MTZ. 1,4-Cyclohexanediol has a chair-like ring structure with two opposite hydroxy groups, which could form H-bonding with adjacent ceramide molecules. Similarly, 1,2-hexanediol has two hydroxy groups which could have H-bonding interactions with both 1,4-cyclohexanediol and ceramide. The



**Fig. 1.** (A) Percentage of the applied dosage in receptor medium at various time points: comparison between F3 and F4 at 750  $\mu\text{g}$  dose. Mean  $\pm$  SD,  $n=6$ . (B) Percentage of the applied dosage in receptor medium at various time points: comparison between F3 and F4 at 375  $\mu\text{g}$  dose. Mean  $\pm$  SD,  $n=6$ . \* $p < 0.05$ , and \*\* $p < 0.01$ . SD: standard deviation.



**Fig. 2.** (A) Percentage of epidermal retention of MTZ: comparison of F3 and F4 at 750 µg dose. Mean ± SD, n = 6. (B) Percentage of epidermal retention of MTZ: comparison of F3 and F4 at 375 µg dose. Mean ± SD, n = 6. SD: standard deviation.

hydrophobic alkyl chain in 1,2-hexanediol could intercalate into the skin lipids.

The width of the ceramide molecule near the circular OH...OH bonds is ~5.8 Å. The size of two packed aliphatic tails is ~8.6 Å (Anishkin et al., 2006). The molecular size of 1,4-cyclohexanediol and 1,2-hexanediol is estimated to ~6.7 Å and ~5.8 Å, respectively.

We postulate that the observed penetration retardation effect is probably due to formation of a hydrogen bonding complex between 1,2-hexanediol and 1,4-cyclohexanediol. This complex could disrupt the SC bilayer due to lipophilic chain in 1,2-hexanediol and carry 1,4-cyclohexanediol into the SC layer. The intercalated 1,4-cyclohexanediol and 1,2-hexanediol complex could form

**Table 4**  
Skin permeation parameters of MTZ at various doses (F5–F6).

Formulation	Dose (µg)	T <sub>lag</sub> (h)	Flux (µg/cm <sup>2</sup> /h)	Amount in collection medium at 24 h (µg)	RR
F5	375	1.08 ± 0.38	1.64 ± 0.25	334.18 ± 8.96	–
F6	375	1.02 ± 0.36	1.67 ± 0.17	322.10 ± 15.33	1.02
F5	750	0.90 ± 0.10	3.80 ± 0.38	628.76 ± 23.28	–
F6	750	0.88 ± 0.12	3.76 ± 0.33	627.96 ± 26.79	0.99

Each value represented the mean ± SD (n = 6).  
RR, retardant ratio. RR = flux for the formulation containing retardation/flux for control formulation.

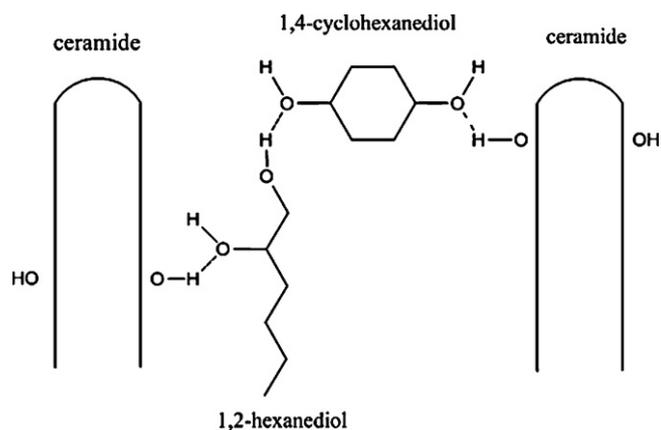


Fig. 3. Schematic of H-bonding interactions between ceramides and retardants. - - : H-bonding.

two-sided H-bonding (H-bond crosslinking) with neighboring ceramide molecules, creating a crosslinked network. This network could condense the SC lipids and make the skin less permeable, leading to penetration retardation of MTZ (see Fig. 3).

Our data have shown that either 1,2-hexanediol or 1,4-cyclohexanediol alone does not act as a penetration retardant. A possible reason is that 1,2-hexanediol by its self could not form H-bond crosslinking, whereas water-soluble 1,4-cyclohexanediol alone might not be lipophilic enough to penetrate the SC layer. The lack of penetration retardation effect with either 1,2-propanediol or 1,4-cyclohexanediol alone provides further evidence that the duo action of skin lipid disruption and two-sided H-bonding is most likely responsible for the retardation effect.

The present study shines light on the mechanistic aspect of the penetration retardation effect. Further studies are currently underway in our laboratories to evaluate other drugs and potential retardant molecules that share similar structural features as the diols on the penetration retardation. The examples include chain length and structure, positions of the OH groups in the diols, or other hydrogen bonding molecules. It is in our plan to study the retardation effect using other skin tissues than hairless mouse skin. These studies will be published in due course.

## 5. Conclusion

In present study, a combination of 1,4-cyclohexanediol and 1,2-hexanediol as penetration retardants for percutaneous absorption and penetration of MTZ has been demonstrated. The presence of both 1,4-cyclohexanediol and 1,2-hexanediol in the formulation significantly reduce the flux values of the applied MTZ without decreasing its retention in dermatologically viable epidermis and dermis layer. This might point to their potential use in dermatological formulations for reducing potential systematic side effect while maintaining therapeutic efficacy. Furthermore, preliminary

evidence suggests that a duo action of skin lipid disruption and H-bond crosslinking might be responsible for the observed retardation effect. Elucidation of the retardation mechanism would help us to discover novel penetration retardants.

## References

- Abraham, M.H., Chdha, H.S., Mitchell, R.C., 1995. The factors that influence skin penetration of solutes. *J. Pharm. Pharmacol.* 47, 8–16.
- Anishkin, A., Sukharev, S., Colombini, M., 2006. Searching for the molecular arrangement of transmembrane ceramide channels. *Biophys. J.* 90, 2414–2426.
- Asbill, C.S., El-Kattan, A.F., Michniak, B., 2000. Enhancement of transdermal drug delivery: chemical and physical approaches. *Crit. Rev. Ther. Drug Carrier Syst.* 17, 621–658.
- Asbill, C.S., Michniak, B.B., 2000. Percutaneous penetration enhancers: local versus transdermal activity. *Pharm. Sci. Technol. Today* 3, 36–41.
- Baker Jr., E.L., Warren, M., Zack, M., Dobbin, R.D., Miles, J.W., Miller, S., Alderman, L., Teeters, W.R., 1978. Epidemic malathion poisoning in Pakistan malaria workers. *Lancet* 1, 31–34.
- Batheja, P., Song, Y., Wertz, P., Michniak-Kohn, B., 2009. Effects of growth conditions on the barrier properties of ahumanskin equivalent. *Pharm. Res.* 26, 1689–1700.
- Briassoulis, G., Narlioglou, M., Hatzis, T., 2001. Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children. *Hum. Exp. Toxicol.* 20, 8–14.
- Catz, P., Friend, D.R., 1990. Transdermal delivery of levonorgestrel. VIII. Effect of enhancers on rat skin, hairless mouse skin, hairless guinea pig skin, and human skin. *Int. J. Pharm.* 58, 93–102.
- Chattaraj, S.C., Walker, R.B., 1995. Penetration enhancer classification. In: Smith, E.W., Maibach, H.I. (Eds.), *Percutaneous Penetration Enhancers*. CRC Press, Boca Raton, FL, pp. 5–20.
- Cross, S.E., Roberts, M.S., 2000. The effect of occlusion on the epidermal penetration of parabens from a commercial test ointment, acetone and ethanol vehicles. *J. Invest. Dermatol.* 115, 914–918.
- Fang, J.Y., Hwang, T.L., Leu, Y.L., 2003. Effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogels. *Int. J. Pharm.* 250, 313–325.
- Goodman, M., Barry, B.W., 1988. Action of penetration enhancers on human skin as assessed by the permeation of model drugs 5-fluorouracil and estradiol. I. Infinite dose technique. *J. Invest. Dermatol.* 91, 323–327.
- Hadgraft, J., Peck, J., Williams, D.G., Pugh, W.J., Allan, G., 1996. Mechanisms of action of skin penetration enhancers/retarders: azone and analogues. *Int. J. Pharm.* 141, 17–25.
- Hoogstraate, A.J., Verhoef, J., Brussee, J., IJzerman, A.P., Spies, F., Boddé, H.E., 1991. Kinetics, ultrastructural aspects and molecular modeling of transdermal peptide flux enhancement by N-alkylazacycloheptanones. *Int. J. Pharm.* 76, 37–47.
- Howes, D., Guy, R., Hadgraft, J., 1996. Methods for assessing percutaneous absorption. The report and recommendations of ECVAM Workshop 13. *Altern. Lab. Anim.* 24, 81–106.
- Kim, N., El-Khalili, M., Henary, M.M., Strekowski, L., Michniak, B.B., 1999. Percutaneous penetration enhancement activity of aromatic S-dimethyliminiosulfuranes. *Int. J. Pharm.* 187, 219–229.
- Lewis, D., Hadgraft, J., 1990. Mixed monolayers of dipalmitoyl phosphatidylcholine with Azone or oleic acid at the air–water interface. *Int. J. Pharm.* 65, 211–218.
- Li, N., Su, Q., Tan, F.P., Zhang, J., 2010. Effect of 1,4-cyclohexanediol on percutaneous absorption and penetration of azelaic acid. *Int. J. Pharm.* 387, 167–171.
- Mancini, A.J., 2004. *Skin. Pediatrics* 113, 1114–1119.
- Potts, R.O., Guy, R.H., 1995. A predictive algorithm for skin permeability: the effects of molecular size and hydrogen bond activity. *Pharm. Res.* 12, 1628–1633.
- Rajadhyaksha, V., Pfister, W.R., 1996. Oxazolidinones. *Drug Cosmet. Ind.* 1, 104–107.
- Roberts, M.S., Pugh, W.J., Hadgraft, J., 1996. Epidermal permeability-penetrant structure relationships: 2. The effect of H-bonding groups in penetrants on their diffusion through the stratum corneum. *Int. J. Pharm.* 132, 23–32.
- Schlumpf, M., Cotton, B., Conscience, M., Haller, V., Steinmann, B., Lichtensteiger, W., 2001. In vitro and in vivo estrogenicity of UV screens. *Environ. Health Perspect.* 109, 239–244.
- Seth, B., 1999. Transdermal delivery using decyloxazolidin-2-one. *Arzneim. Forsch. Drug Res.* 42, 120–122.
- Wertz, P.W., 1992. Epidermal lipids. In: *Seminars in Dermatology*, vol. 11, pp. 106–113.