In Vitro Percutaneous Absorption of Thiamine Disulfide through Rat Skin from a Mixture of Propylene Glycol and Fatty Acid or Its Analog

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Percutaneous absorption of thiamine disulfide, (TDS), a lipophilic derivative of thiamine, from a mixture of propylene glycol (PG) and fatty acid (FA) or its analog through rat skin was tested in vitro. Lauric acid (12:0) enhanced the absorption depending on its concentration in PG and showed a maximal enhancement at 10% w/v. At 10% w/v, lauryl alcohol also enhanced the absorption, but less than 12:0, while lauric acid methyl ester suppressed the absorption. The flux of TDS did not depend on the solubility of TDS in the vehicle, but on the permeability coefficient. From these results, it is suggested that FA increases the permeability coefficient not only because FA increases TDS diffusion by disrupting lipid packing in the stratum corneum but also, FA increases TDS partition to lipid phase by interacting with TDS.

Keywords thiamine; thiamine disulfide; fatty acid; propylene glycol; rat; percutaneous absorption; fatty alcohol; fatty acid methyl ester; permeability coefficient

In stressful modern life, vitamin B1 (thiamine) is necessary for fatigue reduction and nutritional supplementation. We studied thiamine transdermal absorption for the purpose of developing a convenient means of thiamine administration and solving some pharmaceutical problems stemming from thiamine preparations. A thiamine transdermal system may be useful for alcohol-induced thiamine malabsorption causing beriberi and Wernicke’s encephalopathy.1 Thiamine disulfide (TDS), a lipophilic derivative of thiamine, permeates through rat skin from propylene glycol (PG).2 Fatty acid (FA) can be applied to this system as an enhancer. Many studies about the mechanism of percutaneous enhancement by FA have been reported and they describe that FA disrupts lipid packing in the stratum corneum.3–5 In addition to the above effects of FA, an interaction of FA with TDS may be related to the enhanced TDS permeation from the mixture of FA and PG. But, how the interaction affects the TDS permeation is still unknown.

In light of the optimization of the TDS absorption, it is necessary to study the effect of the interaction between TDS and FA on TDS percutaneous absorption. In this report, we tested the enhancing effect of FA on TDS percutaneous absorption as a function of FA concentration in PG, and also evaluated the effect of other FA analogs, methyl ester and alcohol, using rat skin in vitro. The mechanism of the enhancing effect of FA on TDS percutaneous absorption is discussed.

**Experimental**

**Materials** TDS, extra pure grade, and lauric acid methyl ester (12:0 methyl), extra pure grade, were purchased from Tokyo Kasei Industries Co., Ltd. Lauric acid (12:0) was purchased from Sigma Chemical Co., Inc. Myristic acid (14:0), guaranteed reagent grade, and lauryl alcohol (12 OH), extra pure grade, were purchased from Wako Pure Chemical Industries Co., Ltd. Stearic acid (18:0), guaranteed reagent grade, was purchased from Koso Chemical Co., Ltd. PG, Japanese Pharmacopoeia grade, was purchased from Yamada Pharmaceutical Co., Ltd. All other chemicals and solvents were guaranteed reagent grade.

**Animals** Male rats (Std/Flat/DY strain) with a weight of 230 ± 32.8 g were supplied by Sankyo Laboratory Service.

**Skin Membrane Preparation** The abdominal region of the rat was carefully shaved with an electric razor and a hand razor. Two pieces of 2 cm² section of skin with a thickness of 0.512 ± 0.101 mm were excised.

**Skin Permeation Procedure** An excised section of skin was mounted between two half diffusion cells (horizontal) (Nagoya Science Co., Ltd.), each with a volume of 5.0 ml and an effective diffusion area of 0.636 cm². The dermis side of the skin was in contact with a receiver compartment and the stratum corneum with a donor compartment. The receiver compartment of the cell was filled with 5 ml of phosphate buffered saline (pH 7.3) (PBS) and the donor compartment with 5 ml of the drug suspension in vehicle. The donor chamber was sealed from the atmosphere with Parafilm. The diffusion cells were maintained at 37°C in a water bath, and both the donor and receiver compartments were stirred vigorously to equalize the concentration in each cell throughout the experiment. At appropriate times, 200 µl samples were withdrawn from the receiver compartment and assayed for the amount of TDS present. After sampling, 200 µl of PBS was added to the receiver compartment to keep the volume constant. The mixture of PG and 12:0 or 12:0 analog were prepared at 37°C, and clear solutions were obtained for all vehicles. TDS was then added to each vehicle above the amount of solubility in order to maximize the skin permeation. The amount of TDS in the receiver phase was determined by high performance liquid chromatography (HPLC). Propyl p-hydroxybenzoate (PP) was used as an internal standard. The conditions were as follows: pump, HLD-803D (Toyoda Soda); column, 4.0 x 150 mm, Nucleosil 100 SC18 (Gasukuro Industries Co., Ltd.); mobile phase, water/methanol (1:1, v/v); detector, UV-8 model III (Toyoda Soda), 254 nm. Retention times of TDS and PP at a flow rate of 0.8 ml/min were 3.3 and 12.0 min, respectively. Peak areas were calculated using a data treatment computer, Chromatopack C-R1A (Shimazu Seisakusyo).

Throughout the experiments TDS was detected as a single peak.

**Solubility of TDS in the Vehicle** The vehicles were prepared in the same manner as described in the above section except for the vehicles containing 14:0 or 18:0 which were prepared as previously reported.2 An excess amount of TDS was added to each vehicle and stirred at 37°C until the solution attained equilibrium. This solution was quickly filtered through a membrane filter (DISMIC-25SP, PTFE, 0.50 µm, Toyo Roshi) and the filtrate was diluted with Clark–Lubs buffer (pH 1.2). Insoluble FA or FA analog was removed by a cotton filter and then a membrane filter (FH, 0.5 µm, Nihon Millipore Kogyo Co., Ltd.). The amount of TDS in the filtrate was determined spectrophotometrically at 242 nm using the factor 3.89 x 10⁻³ cm² µg⁻¹. The experiment was carried out at least 6 times, and the results were highly reproducible.

**Data Treatment** According to fick’s second law of diffusion, the total amount of penetrant (Q(t)) appearing in the receptor fluid in time t is expressed as follows:

\[ Q(t) = A \times K \times L \times C_0 \times \frac{(1-e^{-D/L})}{L} \]

\[ Q(t) = A \times K \times L \times C_0 \times \frac{1}{L} \]

where A is the effective diffusion area, C₀ is the constant concentration of the donor solution, D is the diffusion constant, L is the thickness of the membrane and K is the partition coefficient of penetrant between membrane and donor solution. When t → ∞ (steady state), Eq. 1 is expressed as

\[ Q(t) = A \times K \times L \times C_0 \times \frac{(1-e^{-1/L})}{L} \]

\[ Q(t) = A \times K \times L \times C_0 \times \frac{1}{L} \]

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From Eq. 2, flux per unit area at steady state, J is expressed as

\[ J = C_0 K_p = C_0 DK/L \]  

(3)

where \( K_p \) is the permeability coefficient. J was determined from the slope of the steady state portion of the amount of penetrant permeated versus time divided by \( A \). \( K_p \) was calculated from the mean values of J and \( C_0 \).

Results and Discussion

Effect of the 12:0 Concentration on TDS Permeation

12:0 shows the largest enhancing effect in FA on TDS permeation from PG through rat skin at a concentration of 10%. The enhancing effect of 12:0 added in PG was measured as a function of the FA concentration. Figure 1 shows the time course of the amount of TDS permeated through rat skin from the mixture of 12:0 and PG. \( J \), \( C_0 \) and \( K_p \) were determined and are shown in Fig. 2. \( J \) increased with an increase in 12:0 concentration and showed a maximum at 10%, namely, the value at 20% was less than that at 10%. \( C_0 \) slightly decreased with an increase in 12:0 concentration. This may suggest the presence of a specific interaction between TDS and 12:0 in PG. TDS interacts with FA in methanol and in ethanol solubility of TDS increases by addition of FA. \( K_p \) increased with an increase in 12:0 concentration up to 10% and the value at 20% was less than 10%. \( K_p \) increased in parallel with \( J \), but \( C_0 \) decreased. Therefore, it is clear that 12:0 increased \( J \) because 12:0 increased \( K_p \). The increase in \( K_p \) may be due to the increase in both \( K \) and \( D \) defined in the Eq. 3. As FA disrupts the stratum corneum lipid packing and decreases diffusional resistance to permeants, diffusion of TDS in the lipid phase is thought to be stimulated depending on the applied concentration of FA. But, the increasing TDS–FA interaction may also affect the partition of TDS. Ogiso and Shintani have reported that FA not only disrupts the packed structure of lipids but forms a complex with propanolol and that the complex partitions into the lipid phase, resulting in enhancement of the percutaneous absorption of the drug.

By increasing 12:0 concentration from 10 to 20%, both \( C_0 \) and \( K_p \) decreased by 13% and \( J \) decreased by 30%. This result shows that the decrease at 20% 12:0 was due to the decrease in both \( C_0 \) and \( K_p \). Many papers have reported the enhancing effect of FA, wherein the enhancing effect of FA was observed to increase with an increase in FA concentration up to a certain concentration and then decrease gradually with a further addition. This decrease is explained as a result of reduction in the amount of the drug dissolved in the vehicle, or the reduction of the skin/vehicle partition coefficient.

Effect of Other 12:0 Analogs on TDS Permeation

Enhancing effects of fatty alcohol and fatty acid methyl ester

Fig. 2. Effect of 12:0 Concentration in PG on \( J \), \( C_0 \), and \( K_p \) of TDS

Points and vertical bars represent means and the S.D., respectively. The values for r represent relative value of each parameter vs. PG alone.

Fig. 3. Effect of 12:0 Analogs on the Percutaneous Absorption of TDS from PG through Excised Rat Skin

Additive: ○, none; ●, 12:0; ◆, 12-OH; ●, 12:0 methyl. They were added to PG at 10% w/v. Points and bars represent means and S.D., (n=3), respectively.
which have the same carbon number as 12:0 were evaluated at 10% to know the effect of the difference of these polar groups on TDS permeation. Figure 3 shows the time course of the amount of TDS permeated through rat skin from the vehicle. From these results, J, C₀ and Kₚ were determined and illustrated in Fig. 4. 12 OH enhanced the permeation of TDS but less than 12:0, while 12:0 methyl suppressed the permeation. 12:0 methyl did not affect C₀ very much, while 12 OH decreased C₀ more than 12:0. Kₚ values were large in the order of 12:0 > 12 OH > control > 12:0 methyl. 12 OH increased Kₚ, but decreased C₀, and increased J, therefore, the increase in J is due to the increase in Kₚ as found with 12:0. It is clear that the larger J with 12:0 was due to the larger Kₚ when compared with 12 OH. On the other hand, 12:0 methyl decreased J (r = 0.69) accompanying decreased C₀ and Kₚ (r = 0.98, 0.71, respectively). This result shows that the decrease in J by 12:0 methyl resulted from the decrease in Kₚ. Yamada and Uda⁹) have reported the enhancing effect of 12:0 analogs on molsidomine permeation through rat skin from PG is large in the order 12 OH > 12:0 > 12:0 methyl. Ogiso and Shintani⁹) have reported that the effect of the functional group on propranolol absorption from the mixture of PG, ethanol and Carbopol1934 through rat skin is large in the order of 12:0 > 12:0 methyl > control. These results agree with our present results with 12:0 and 12 OH, but not with 12:0 methyl. The enhancing effect of 12 OH is thought to be mainly due to its lipid fluidization effect. Fatty alcohols interact with phospholipids at the boundary lipid layer by hydrophobic interaction, but less than their acid analogs, and this leads to less membrane fluidization effect of alcohols than acids.¹¹) Aliphatic esters are reported to show their percutaneous enhancing effect because of increased lipid fluidization.¹²,¹³) However, the value obtained for Kₚ of 12:0 methyl was smaller than the control. This may be due to the fact that 12:0 methyl decreases TDS partition although it increases diffusion. The decrease in TDS partition might be explained by the interaction ability of 12:0 methyl with TDS. We have previously reported that FA and fatty alcohol interacts with cycotiamine, a lipophilic thiamine derivative, in 1,2-dichloroethane, while fatty acid methyl ester does not.¹⁴) The process where TDS partition increases by the addition of FA or fatty alcohol is thought to include the interaction of TDS with these additives, in addition to their lipid perturbation in the stratum corneum.

**Effect of FA with Different Carbon Numbers** Figure 5 shows the effect of 10% FA on C₀ and Kₚ depending on its carbon number (Cₚ), compared with J previously reported.²) The addition of 14:0 and 18:0 decreased C₀ as well as 12:0. The value of C₀ decreased by increasing Cₚ, 14:0 increased Kₚ, but decreased C₀, and increased J. Therefore, it is clear that 14:0 increased J because 14:0 increased Kₚ as found with 12:0. 18:0 decreased C₀ (r = 0.7), Kₚ (r = 0.89), and J (r = 0.64). The decrease in J by 18:0 is probably affected more by C₀ than Kₚ. The decrease in Kₚ by increasing C₀ may be related to the decreasing solubility of FA in PG by increasing Cₚ, as well as the lipid disrupting effect of FA which depends on Cₚ.¹⁰) As previously reported,²) 10% 12:0 can be solubilized in PG, but 10% 14:0 and 18:0 can not be completely solubilized. Cooper¹⁵) reported that the low solubility of FA in PG restricts the FA effect on transport. The suspended molecules of FA may not be able to partition into skin nor interact with TDS resulting in no increase in TDS partition.
and the enhancing effect of FA may be less than that expected at the FA concentration applied. The capacity of 18:0 and stearic acid methyl ester to stabilize human erythrocytes against hypotonic hemolysis is low, because they mostly exist as aggregates in suspension rather than in their solubilized form owing to low solubility in the test solution, and very few free molecules reach the membrane to stop hemolysis.\textsuperscript{16} The decreasing solubility of FA in PG by increasing $C_0$ might have affected the TDS permeability coefficient, namely partition and diffusion of TDS, as well as the solubility of TDS in vehicle.

**Conclusion**

Figure 6 shows the relationship between $J$ and $C_0$ or $K_p$. A very good correlation was obtained between log $K_p$ and log $J$ (its correlation coefficient is 0.999), whereas little correlation between log $C_0$ and log $J$ was found. From these results, we conclude that TDS flux across rat skin in the FA–PG mixture system primarily depends on TDS skin permeability coefficient. FA increases TDS flux by increasing TDS skin permeability coefficient. This may be attributed to the increased TDS partition to lipid phase in the stratum corneum, probably owing to the interaction between TDS and FA, as well as the increased TDS diffusion induced by lipid perturbation by FA.

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**References**