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

Yasuko KOMATA,* Akiko KANEKO and Tadao FUJIE

Kyoritsu College of Pharmacy, Shibakoen 1–15–30, Minato-ku, Tokyo 105, Japan. *Received March 11, 1992*

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 B_1 (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/Wistar/ST strain) with a weight of 230.2 ± 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 $2\,cm^2$ 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 $0.636\,cm^2$. 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 (Toyo Soda); column, 4.0×150 mm, Nucleosil 100 5C18 (Gasukuro Industries Co., Ltd.); mobile phase, water/methanol (1:1, v/v); detector, UV-8 model III (Toyo 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 previous 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-25JP, 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 $3.89 \times 10^{-5}\,cm^2\,\mu g^{-1}$. 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:

$$Q_t = AKLC_0[D/(L^2)t - 1/6 - 2/\pi^2 \sum_{n=1}^{\infty} (-1)^n/n^2 \exp(-D/(L^2)n^2\pi^2 t)] \quad (1)$$

where A is the effective diffusion area, C_0 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 $t \to \infty$ (steady state), Eq. 1 is expressed as

$$Q_t = AKLC_0(D/(L^2)t - 1/6) \quad (2)$$

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

$$J = C_0 K_p = C_0 DK/L \quad (3)$$

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%.[2] 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,[6] and in ethanol solubility of TDS increases by addition of FA.[7] 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,[5] 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[8] have reported that FA not only disrupts the packed structure of lipids but forms a complex with propranolol 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,[9] or the reduction of the skin/vehicle partition coefficient.[10]

Effect of Other 12:0 Analogs on TDS Permeation Enhancing effects of fatty alcohol and fatty acid methyl ester

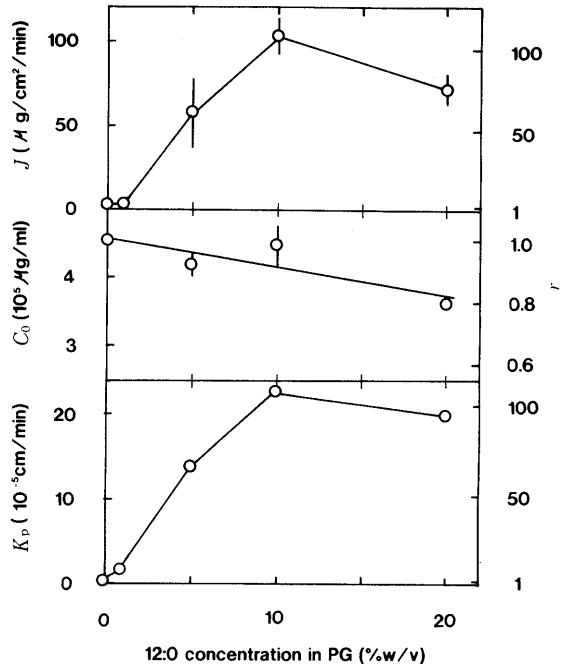

Fig. 2. Effect of 12:0 Concentration in PG on J, C_0, and K_p of TDS

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

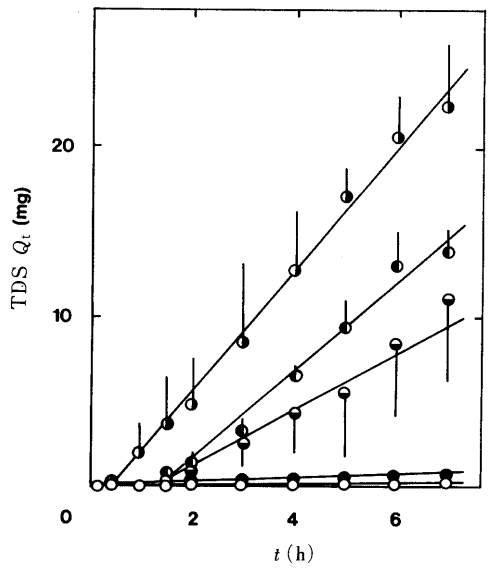

Fig. 1. Effect of 12:0 Concentration in PG on the Percutaneous Absorption of TDS through Excised Rat Skin

Concentration (w/v%): ○, 0; ●, 1; ◒, 5; ◑, 10; ◐, 20. Points and vertical bars represent means and S.D. ($n=3$), respectively.

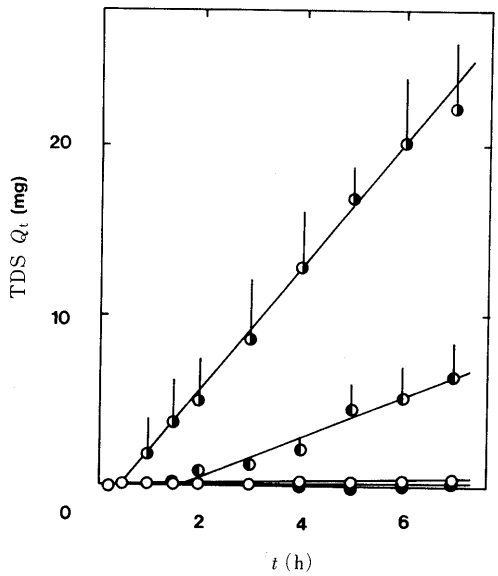

Fig. 3. Effect of 12:0 Analogs on the Percutaneous Absorption of TDS from PG through Excised Rat Skin

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.

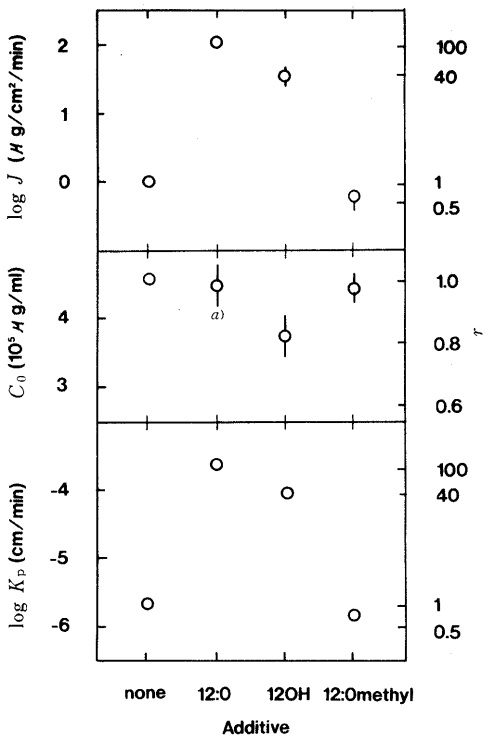

Fig. 4. Effect of 12:0 Analogs on J, C_0 and K_p of TDS

Points and vertical bas represent means and S.D., respectively. The values for r represent the relative value of each parameter *vs.* PG alone. *a*) The value determined from Fig. 2.

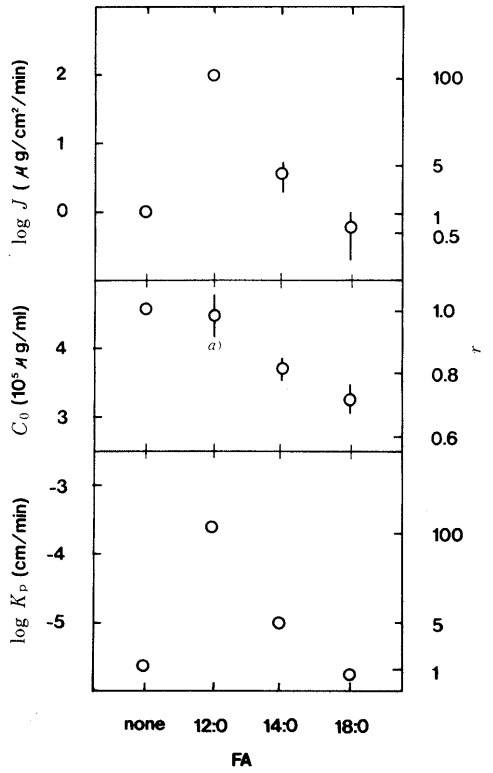

Fig. 5. Effect of Carbon Number of FA on J,[2] C_0 and K_p of TDS

Points and vertical bars represent means and S.D., respectively. The values for r represent the relative value of each parameter *vs.* PG alone. *a*) The value determined from Fig. 2.

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, C_0 and K_p are 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 C_0 very much, while 12 OH decreased C_0 more than 12:0. K_p values were large in the order of 12:0 > 12 OH > control > 12:0 methyl. 12 OH increased K_p, but decreased C_0, and increased J, therefore, the increase in J is due to the increase in K_p as found with 12:0. It is clear that the larger J with 12:0 was due to the larger K_p when compared with 12 OH. On the other hand, 12:0 methyl decreased J ($r=0.69$) accompanying decreased C_0 and K_p ($r=0.98$, 0.71, respectively). This result shows that the decrease in J by 12:0 methyl resulted from the decrease in K_p. Yamada and Uda[9] 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 Shintani[8] have reported that the effect of the functional group on propranolol absorption from the mixture of PG, ethanol and Carbopo1934 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 K_p 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 C_0 and K_p depending on its carbon number (C_n), compared with J previously reported.[2] The addition of 14:0 and 18:0 decreased C_0 as well as 12:0. The value of C_0 decreased by increasing C_n. 14:0 increased K_p, but decreased C_0, and increased J. Therefore, it is clear that 14:0 increased J because 14:0 increased K_p as found with 12:0. 18:0 decreased C_0 ($r=0.7$), K_p ($r=0.89$), and J ($r=0.64$). The decrease in J by 18:0 is probably affected more by C_0 than K_p. The decrease in K_p by increasing C_n may be related to the decreasing solubility of FA in PG by increasing C_n, as well as the lipid disrupting effect of FA which depends on C_n.[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. Cooper[15] 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,

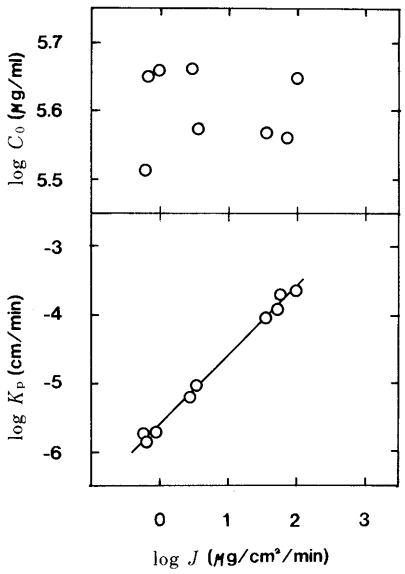

Fig. 6. Relationship between C_0, K_p and J
Points represent the means of each experiment.

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.[16] The decreasing solubility of FA in PG by increasing C_n 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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