Effect of Fatty Acid on the Accumulation of Thiamine Disulfide in Rat Skin

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The effect of long chain fatty acid (FA) and its analogs on the accumulation of thiamine disulfide (TDS) in rat skin using propylene glycol as a vehicle was studied *in vitro*. Lauric acid (12:0) increased the accumulation of TDS in skin, while myristic acid and stearic acid caused a slight decrease in accumulation. Lauryl alcohol and lauric acid methyl ester did not change the accumulation of TDS in the skin. The ratio of the amount of TDS accumulated in skin to the solubility of TDS in the vehicle increased dependent on the concentration of 12:0 added in the vehicle. It was suggested that the increase in the permeability coefficient of TDS by 12:0 results from the enhanced transport of TDS from the vehicle to skin.

Keywords thiamine disulfide; accumulation; skin; fatty acid; propylene glycol

Thiamine disulfide (TDS), an oxidized dimer of thiamine, is readily reduced to thiamine, and shows activity in animals.¹⁾ We have been studying the percutaneous absorption of TDS *in vitro* for the purpose of developing a transdermal system. This system would be applicable to the thiamine deficiency caused by thiamine intestinal malabsorption, which is found as a result of chronic alcohol consumption.²⁾ We believe this administration system may also avoid some of the disadvantages reported following the oral administration or intravenous injection of thiamine such as bioinequivalency³⁾ and tenderness. TDS permeates rat skin using propylene glycol (PG) as a vehicle; long chain fatty acids (FAs) enhance or suppress permeation depending on the number of carbons; lauric acid (12:0) acts as an enhancer.⁴⁾ These effects of FAs can be explained as changes in the permeability coefficient of TDS in the vehicle rather than changes in the solubility of TDS in the vehicle.⁵⁾

In optimizing the transdermal system for TDS, it is interesting to study the mechanism by which the percutaneous permeation of TDS is changed by FA. For this purpose, we determined the amount of TDS accumulated in skin in this report. The mechanism by which FA changes the permeation of TDS is discussed with relation to the permeability coefficient.

MATERIALS AND METHODS

Materials TDS, extra pure grade, and lauric acid methyl ester (12:0 methyl), extra pure grade, were purchased from Tokyo Kasei Industries Co., Ltd. 12:0 was purchased from Sigma 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 251 ± 19 g were supplied by Sankyo Laboratory

Service.

Skin Permeation Procedure The procedure was carried out as reported previously.^{4,5)} A piece of excised abdominal 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 was filled with 5 ml of drug suspension in the vehicle. The donor chamber was sealed from the atmosphere with Parafilm. The diffusion cell was 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 μ l samples were withdrawn from the receiver cell and assayed for the amount of TDS present. After sampling, 200 μ l of PBS was added to the receiver compartment to keep the volume constant. The vehicles used were either PG, PG with FA, or FA analog. They were clear solutions under the experimental condition used, except for those containing 14:0 or 18:0. TDS (2.5 g, an amount greater than its solubility) was added to 5 ml of each vehicle in order to maximize skin permeation.

At the end of the above experiment, the skin was removed from the diffusion cell, washed with PG and water, and wiped off with soft paper towels. The applied area of the skin was excised carefully with scissors, placed in a test tube with 3 ml of PBS, and homogenized (Polytron, Kinematica GmbH, Littau). Five milliliters of methanol was added to this solution, and the mixture was allowed to sit for about 17 h to extract TDS from the skin. After centrifugation, the supernatant was filtered (Dismic-25JP, 0.5 μ m, Toyo Roshi Co., Ltd.), and the filtrate was assayed for the amount of TDS accumulated in the skin.

Determination of TDS The amount of TDS was determined by high performance liquid chromatography using methyl *p*-hydroxybenzoate as an internal standard. The conditions were as follows: pump, HLD-803D (Toyo Soda Co., Ltd.); column, 4.0 $\times$ 150 mm, Nucleosil 100

5C18 (Gasukuro Industries Co., Ltd.); mobile phase, methanol-water (15:85) containing 5 mM sodium hexanesulfonate, 1% acetic acid, and 0.1% triethylamine at a pH of 3.2; detector, UV-8 model III (Toyo Soda), 254 nm. The retention times of TDS and methyl *p*-hydroxybenzoate at a flow rate of 1.1 ml/min were 4.9 min and 12.2 min, respectively. Peak areas were calculated using a data treatment computer, Chromatopack C-R1A (Shimazu Seisakusyo). Throughout the experiment TDS was detected as a single peak.

Solubility of TDS in the Vehicle The vehicles were prepared in the same manner as for the permeation procedure. An excess amount of TDS was added to each vehicle and stirred at 37°C for 5 h. This solution was quickly filtered through a membrane filter (Dismic-25JP, 0.5 μ m, Toyo Roshi Co., Ltd.), and the filtrate was diluted with Clark-Lubs buffer (pH 1.2). Insoluble FA was removed with filter paper. The amount of TDS in the filtrate was determined spectrophotometrically at 242 nm, using $\epsilon = 2.19 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$. Experiments were carried out at least 6 times, and the results were highly reproducible.

Solubility of TDS in Isopropyl Myristate An excess amount of TDS was added to a FA solution in isopropyl myristate. The solution was stirred at 25°C until it attained equilibrium. The solution was centrifuged and the supernatant was assayed for TDS by HPLC. All experiments were carried out at least 3 times.

RESULTS AND DISCUSSION

Figure 1 shows the time course for the cumulative amount of TDS permeating through the skin from PG with and without 5% (w/v) FA. The presence of 12:0

Fig. 1. Effect of the Number of Carbons in FA on the Percutaneous Absorption of TDS from PG through Excised Rat Skin

FA: ●, 12:0; ○, 14:0 and control; ●, 18:0.
FA was added to PG at 5% (w/v). Points and bars represent means and S.D., ($n=3$), respectively.

enhances the permeation of TDS from PG, but 14:0 has little effect, and 18:0 suppresses permeation. This dependency on the number of carbons in the FA is similar to that obtained with 10% FA (12:0 > 14:0 > 18:0).⁴⁾ The values for flux per unit area at steady-state (J) were determined from Fig. 1, and permeability coefficients (K_p) were estimated using the equation:

$$J = C_0 K_p$$

where C_0 is the constant concentration of the donor solution, and the solubility of TDS in each vehicle was used as this value. These values are compared with those for 10% FA⁵⁾ in Fig. 2. The change in J , which decreases as the carbon number increases, is affected more by K_p than C_0 .⁵⁾ In the case of 14:0, as with 12:0, the K_p at 5% was smaller than that at 10%. On the other hand, the K_p at 5% 18:0 was larger than at 10%. Therefore, the enhancing or suppressing effect of each FA at 5% was smaller than at 10%, and the dependency of K_p on the number of carbons in the FA at 5% was similar to that at 10%.⁵⁾

Figure 3 shows the accumulation of TDS when 5% FA is added to the vehicle. The accumulation of TDS in skin was increased by 12:0, but 14:0 and 18:0 caused a slight decrease. As shown in Fig. 2, FA changes the solubility of TDS in the vehicle, and to take this effect into consideration, the ratio of the amount accumulated in skin to C_0 (TDS in skin/ C_0) was calculated (Fig. 3). The ratio was increased by 12:0, while 14:0 and 18:0 caused a slight decrease. Thus, it was suggested that the difference in concentration of TDS between skin and vehicle PG was

Fig. 2. Effect of the Number of Carbons in FA on the J , C_0 and K_p of TDS

The concentration of FA in PG: ○, 5%; ●, 10%.⁴⁾

Fig. 3. Effect of the Number of Carbons in FA on the Accumulation of TDS in Excised Rat Skin

FA was added to PG at 5% (w/v). Points and bars represent means and S.D., ($n=3-4$), respectively.

Fig. 4. Effect of 12:0 Analogs on K_p ⁵⁾ and the Accumulation of TDS in Excised Rat Skin

Each FA analog was added to PG at 10% (w/v). Points and bars represent means and S.D., ($n=3-4$), respectively.

increased by 12:0, while slightly decreased by 14:0 and 18:0.

Figure 4 shows the effect of FA analogs on the accumulation of TDS in the skin. Unlike 12:0, 12 OH and 12:0 methyl did not increase the accumulation of TDS in skin. The values for TDS in skin/ C_0 suggest that these analogs barely change the difference in concentration of TDS between skin and vehicle. As can be seen in both Figs. 3 and 4, the variation of TDS in skin/ C_0 is similar

TABLE I. Effect of FA and Its Analogs on the Solubility of TDS in Isopropyl Myristate at 25°C

FA	Solubility ($\times 10^{-5}$ M)
None	0.99 ± 0.27
18:0	3.11 ± 1.33
14:0	3.10 ± 0.32
12:0	2.57 ± 1.34
12 OH	1.61 ± 0.89
12:0 methyl	0.78 ± 0.37

The concentration of each FA analog was 1×10^{-2} M.

Fig. 5. Effect of 12:0 Concentration in PG on K_p ⁵⁾ and the Accumulation of TDS in Excised Rat Skin

Points and bars represent means and S.D., ($n=3-4$), respectively.

to that of TDS in skin, suggesting that the changes in the accumulation in skin by these adjuvants are greater than those in C_0 .

The mechanisms for increasing the amount of drug in skin have been reported; the increased solvent transport, which results from a decrease in diffusion resistant by adjuvants, concomitantly causes the permeation of drug⁶⁾; the formation of a lipophilic ion pair between the drug and the adjuvant increases the partition of drug to skin.⁷⁾ The ability of FAs to increase the fluidization of the stratum corneum when PG is used as a vehicle has been quantitatively evaluated by Takeuchi, *et al.*,⁸⁾ and they state that this ability decreases corresponding to the increase in the number of carbons in FA from 12 to 18, with no effect by 18:0. They also state that the carboxyl groups of FAs are not determinant factors for this effect. This report can explain the accumulation of TDS dependent on the number of carbons in FA, but not the variations by FA analogs. Table I shows the effect of FA and its analogs on the solubility of TDS in isopropyl

myristate. Isopropyl myristate is often used as a model of skin constituents.⁹⁾ The increased solubility of TDS by the addition of FAs and alcohol (12:0 > 12 OH) suggests the possible formation of complexes between TDS and these adjuvants in skin. However, these results can still explain only the effect of each FA analog. Eventually, neither of these theories can explain the effects of these adjuvants alone, thus a complex mechanism involved in changing the accumulation of TDS in skin is assumed. But, at least, it is thought that the significant increase in accumulation of TDS by 12:0 is related to the larger value for K_p .

Figure 5 shows the accumulation of TDS in skin relative to the concentration of 12:0 in PG. The accumulation of TDS increased with an increase in the concentration of 12:0, but leveled off above concentrations of 5%. The value for TDS in skin/ C_0 increased as the concentration of 12:0 increased, even above the concentration of 5%, although slightly. It suggests that the difference of concentration of TDS between skin and vehicle increases proportionately to the concentration of 12:0 in the vehicle. This result agrees with the variation of K_p dependent on the concentration of 12:0. Thus, it is thought that the increase in K_p by 12:0 results from the enhanced transport of TDS from vehicle to skin.

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