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Effect of phosphatidylglycerol on the in vitro percutaneous drug penetration through the dorsal skin of guinea pigs, and analysis of the molecular mechanism, using (ATR-FTIR) spectroscopy

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Abstract

Standard in vito skin percutaneous penetration methods using excised guinea pig dorsal skin were employed, to characterize the penetration of a water-insoluble drug: tenoxicam (TEX), and a water-soluble drug: diclofenac sodium salt (DFS), enhanced by phosphatidylglycerol (PG); and an attenuated total reflectance fourier transform infrared (ATR-FTIR) spectroscopy was used to analyze the molecular mechanism of the drug penetration route. The C–H bond stretching absorbance frequency shift in the stratum corneum (SC) induced a higher and a broader absorbance, and the shift was dependent on the PG concentrations. The percutaneous penetration of TEX was dependent on the PG concentration (up to 6%). The enhancing mechanism of PG to TEX may not only increase the diffusion coefficient (D) and the partition coefficient (K) in the percutaneous TEX penetration but also increase fluidity of the route (intercellular lipid domain) for TEX, while that of PG to DFS, excepting 1% PG system, may be increasing the D value in the percutaneous DFS penetration only. The percutaneous penetration of DFS was not dependent on the PG concentrations. Furthermore, the percutaneous penetration of TEX was proportional to the C–H bond stretching absorbance frequency shifts. In contrast, the percutaneous penetration of DFS was not proportional to the C–H bond stretching absorbance frequency shifts. Furthermore, the accumulation of TEX in skin was proportional to the C–H bond stretching absorbance frequency shifts. The striking parallel between the enhancement of the percutaneous penetration of TEX and the measured SC lipid fluidity shifts caused by PG, suggests that the transdermal water-insoluble drug penetration may be ultimately related to the SC lipid structure. Overall, these results suggest that PG mainly affects the intercellular lipid pathway (lipid-rich domains). © 1997 Elsevier Science B.V.

Keywords: Percutaneous penetration; Phosphatidylglycerol; ATR-FTIR spectroscopy; Tenoxicam; Diclofenac sodium salt

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1. Introduction

The primary barrier to transdermal drug diffusion is the stratum corneum (SC), the thin outermost layer of the skin that is comprised of a regular array of protein-rich cells embedded in a multilamellar lipid domain (Golden et al., 1987). The lipid-protein-partitioning theory of skin penetration enhancement suggested that enhancers had usually two main mechanisms; they could alter the intercellular lipid or intracellular protein domains of the SC, and might also increase partitioning into the skin of drugs (Barry, 1991). One of the major limitations of such enhancers is their potential toxicity and skin irritancy (Hadgraft, 1989). As such, enhancers that are safe for use on the skin are desired. We previously focused on phospholipids that are degraded in the skin. In a previous study, we investigated, systematically, the effects of a series of phospholipids that have different hydrophilic groups (Yokomizo and Sagitani, 1996a) and different hydrophobic groups (Yokomizo and Sagitani, 1996b), on the percutaneous, penetration of indomethacin, in vitro. The varied degree of enhancement by phospholipids niay be due to both differences of hydrophilic and hydrophobic groups. Further, phospholipid having unsaturated acyl chains may be effective penetration enhancers for the percutaneous delivery of certain topically applied drugs (Yokomizo, 1996). Furthermore, we suggested that phospholipids directly influenced the SC on the skin surface (Yokomizo and Sagitani, 1996a). Microscopic and biophysical probing of the SC lipid structure has revealed a well-ordered, lamellar organization (William and Elias, 1987; White et al., 1988). At physiological temperatures, FT-IR spectroscopic results indicated that certain enhancers caused SC lipid disorder. Since these same promoters enhanced drug flux across the skin, the hypothesis was proposed that the enhancement mechanism was lipid 'fluidization'. Further, the ATR-IR method can also measure other constituents of the delivery system (cosolvents, enhancers, etc.) along with changes in SC lipid and protein absorbances. The purpose of this study was to clarify the effects of PG on the percutaneous penetration routes of using a water-insoluble drug (TEX) and a water-soluble drug (DFS) through the dorsal skin of guinea pigs, in vitro. and we further analyzed the mechanism by which PG enhances the lipid fluidity of the guinea pig SC, using an ATR-FTIR spectroscopy, in vitro.

2. Materials and methods

2.1. Reagent

Phosphatidylglycerol (PG; trade name, Phospholipids-PGE; purity, 95.7%) from egg yolk was purchased from Nippon Fine Chemical Co. Ltd. (Tokyo, Japan). Tenoxicam (TEX) and diclofenac sodium salt (DFS) were supplied by Sigma (MO, USA). Kanamycin sulfate was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Acetonitrile, methanol, distilled water, disodium hydrogenphosphate 12-water, and triethylamine for HPLC were obtained from Wako Pure Chemical Industries Ltd. Tween-80 and propylene glycol were purchased from Nikko Chemical Co. Ltd. (Tokyo, Japan). Phosphate-buffered salts (Dulbecco's formula, modified without magnesium and calcium) was supplied by Takara Co. Ltd. (Tokyo, Japan).

2.2. Animal

Male Hartley strain guinea pigs (body weight, 250–300 g) were purchased from Seibutsu Igaku Research Center (Kanagawa, Japan). They were housed in temperature-controlled rooms (25 ± 2°C), with a 12-h light/dark cycle (7:00–19:00 h), and they were allowed free access to food (M.F. Oriental) and tap water for 1 week. The experiment of percutaneous penetration was carried out using excised dorsal skin in a water bath at 37°C.

2.3. Percutaneous penetration of drugs through the guinea pig dorsal skin and accumulation of drugs in skin, in vitro

The skin diffusion experiment was carried out using a Franz-type diffusion cells as described previously (Yokomizo and Sagitani, 1996a). The dorsal fur of male guinea pigs (body weight,

300–350 g) was removed carefully with 0.1 mm hair clippers. The dorsal skin was excised after the animals were sacrificed, and the subcutaneous fat was eliminated. The whole skin (epidermis and deris) was used as experimental skin, and it was mounted on a 50-ml Franz-type diffusion chamber. The donor area was 3.14 cm^2, on which 1 g of test solution was put in contact with the epidermis. The receptor volume was 50 ml, and to maintain physiological sink conditions, the dermal side was continuously kept in contact with 50 ml of phosphate buffer solution at pH 7.4. The phosphate buffer solution contained 0.5% (w/v) Tween-80, to dissolve penetrated drugs, and 100 ppm of kanamycin sulfate, to avoid bacterial proliferation. Four experiments were carried out in each system at 37°C. The vehicle used for diffusion experiments was usually propylene glycol containing 1% (w/w) of drugs. Phospholipids were also dissolved in vehicle. After 1 g of vehicle had been applied to each skin sample, 1 ml samples of receptor fluid were withdrawn periodically and replaced with the same volume of buffer solution. At the end of the diffusion experiment, the drugs and PG remaining in the donor cell and in the skin was recovered as described previously (Yokomizo, 1996).

2.4. Analytical method for tenoxicam (TEX), diclofenac sodium salt (DFS), and phosphatidylglycerol (PG)

Analytical methods for model drugs and a phospholipid (phosphatidylglycerol) were carried out using HPLC systems as described previously (Yokomizo, 1996 in press).

2.5. Determination of diffusion coefficient (D) and partition coefficient (K) of drugs, by using computer simulations

The determination of diffusion coefficient (D) and partition coefficient (K) were determined using a computer simulation as described previously (Yokomizo and Sagitani, 1996c), based on the nonlinear least-squares computer program: MULTI (Yamaoka et al., 1981).

2.6. Preparation of skin for ATR-FTIR spectroscopy

The dorsal fur of male guinea pigs (body weight, 300–350 g) was removed carefully with 0.1 mm hair clippers. The dorsal skin was excised after the animals were sacrificed, and the skin was cut to a size of about 2 × 10 cm. The whole skin (epidermis and dermis) was employed as experimental skin. The epidermal side of the skin was pretreated with some concentrations of PG-propylene glycol solution for 48 h, and the skin was wiped briefly with Kimwipes, containing propylene glycol prior to the experiment. The remaining amount of PG on the skin was almost removed, not disturbing an absorbance peak derived from intercellular lipids in the SC.

2.7. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy study

A spectrum of guinea pig SC that had been treated with PG was measured, in vitro. In the present study, a JEOL JIR-5500 Fourier-transform infrared spectrophotometer (Tokyo, Japan) was used to record the in vitro measurement. The spectrophotometer was equipped with an ATR accessory (Skin Analyzer, Spectra Tech, Stanford, CT), which supported a zinc selenide (7.5 × 1 × 0.2 cm, with a 45° incident angle). This ATR configuration results in a penetration depth of the IR beam of approximately 2 μm. All spectra reported were the average of ten scans (requiring a data collection period of about 20 s). Location of the IR absorbance-peak maximum was determined to an accuracy of 0.1 cm^{-1} using a center-of-gravity algorithm. Measurements were made at ambient laboratory conditions (relative humidity, 50–60%; temperature, 25 ± 1°C).

2.8. Statistical analysis

The results are expressed as the mean ± standard deviation. Statistical analysis was carried out using Student's *t*-test. Significant differences were expressed at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

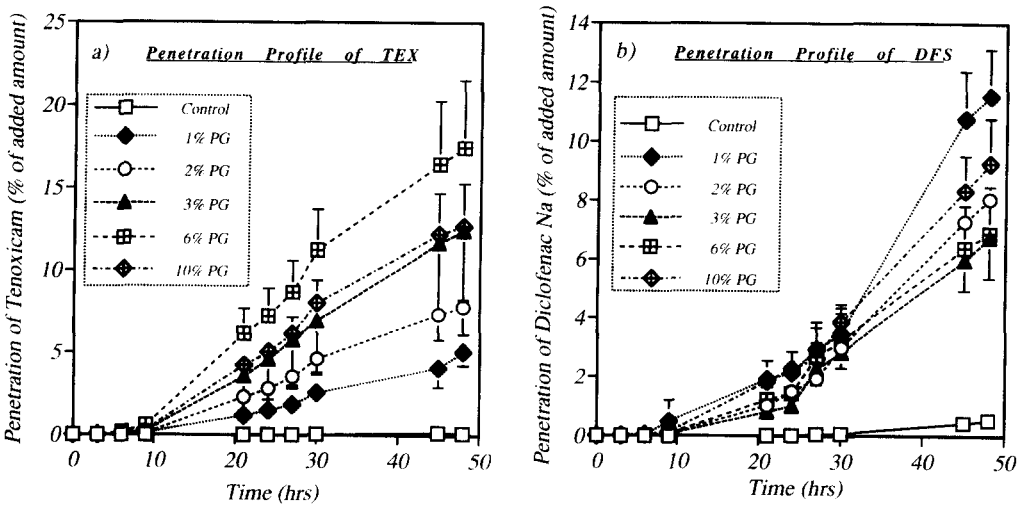

Fig. 1. (a) Effects of PG on the percutaneous penetration profile of TEX through the dorsal skin of guinea pigs, in vitro. Concentrations of PG are 0%, 1%, 2%, 3%, 6%, and 10%. (b) Effects of PG on the percutaneous penetration profile of DFS through the dorsal skin of guinea pigs, in vitro. Concentrations of PG are 0%, 1%, 2%, 3%, 6%, and 10%.

3. Results and discussion

3.1. Effect of PG on the percutaneous penetration profile of drugs: in vitro study

Fig. 1a shows the effects of PG on the percutaneous penetration profile of TEX through the dorsal skin of guinea pigs, in vitro. This result indicates that the percutaneous TEX penetration is increased significantly in the presence of PG, at all times and PG concentrations. Further, the percutaneous penetration profile of TEX, enhance by PG is dependent on the PG concentration, up to 6% PG. Fig. 1b shows the effects of PG on the percutaneous penetration profile of DFS through the dorsal skin of guinea pigs, in vitro. This result suggests that the percutaneous DFS penetration is increased significantly in the presence of PG, at all times and PG concentrations. In addition, the percutaneous penetration profile of DFS, enhance by PG is not dependent on the PG concentration. These results suggests that the different of the percutaneous penetration ability of PG to the hydrophilic and hydrophobic drug penetration, may be the reason for that of penetration routes of both drugs (TEX, lipid pathway: DFS, polar pathway). Furthermore, the effect of PG on the lag time of the percutaneous penetration profile of drugs is a little (Fig. 1a and b), so PG is considered to be a mild penetration enhancer for skin.

3.2. Percutaneous penetration of drugs: in vitro study

Fig. 2a shows the relationship between the PG concentration and the percutaneous penetration of TEX through the dorsal skin of guinea pigs after 24 h and 48 h, in vitro. The maximum accumulated arnount of TEX passing to the receptor side increased significantly, approximtely 100-fold ($P < 0.01$: in the presence of 6% PG), compared to the control. The enhancing effect of PG on the percutaneous penetration of TEX is proportional to the concentration of PG, up to the concentration of 6%. The correlation coefficient obtained for the parabolic curve fit of the percutaneous TEX penetration amount versus the dose of PG, is high: $r = 0.991$ (after 24 h) and $r = 0.996$ (after 48 h). Table 1 shows the effect of PG on the value of D and K in the percutaneous TEX penetration, by computer simulation. Both the value of D and K in the percutaneous TEX penetration are significantly increased in the presence of PG. The enhancing effect of PG on the

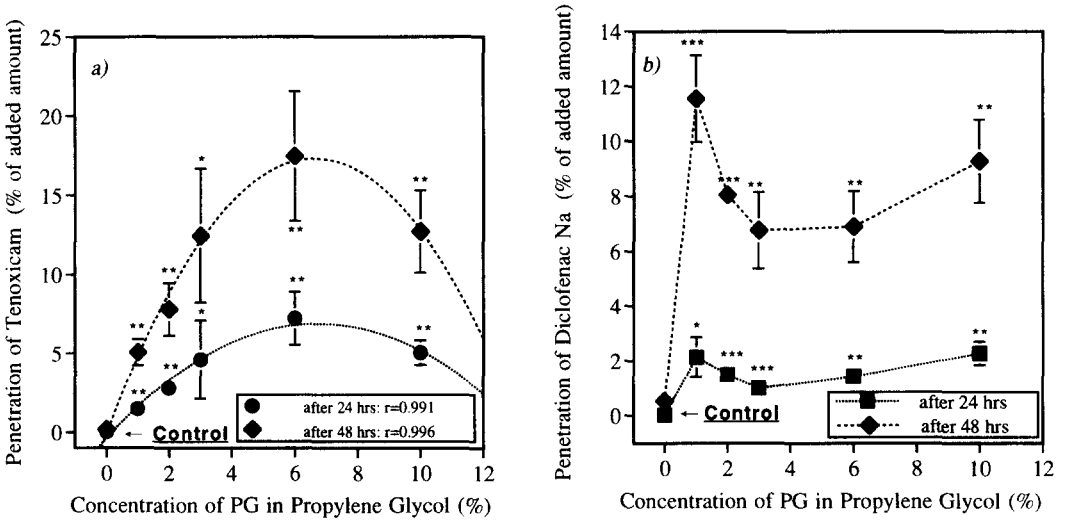

Fig. 2. (a) Relationship between the PG concentration and the percutaneous penetration of TEX through the dorsal skin of guinea pigs after 24 h and 48 h, in vitro. Data are the mean ± S.D. ($n = 3$–4). * Significantly different from control at $P < 0.05$. ** Significantly different from control at $P < 0.01$. (b) Relationship between the PG concentration and the percutaneous penetration of DFS through the dorsal skin of guinea pigs after 24 h and 48 h, in vitro. Data are the mean ± S.D. ($n = 3$–4). ** Significantly different from control at $P < 0.01$. *** Significantly different from control at $P < 0.001$.

percutaneous penetration of TEX is proportional to the concentration of PG, up to the concentration of 6%. Because, the value of D and K in the percutaneous TEX penetration are proportional to the concentration of PG, up to 6%. Further, this result suggests that it may be reason for decreasing the release rate of TEX from vehicles, in the presence of 10% PG more than 6% PG system. Fig. 2b shows the relationship between the PG concentration and the percutaneous penetration of DFS through the dorsal skin of guinea pigs after 24 h and 48 h, in vitro. The maximum accumulated amount of DFS passing to the receptor side increased significantly, approximately 22-fold ($P < 0.001$: in the presence of 1% PG), compared to the control. The enhancing effect of PG on the percutaneous penetration of DFS was not proportional to the concentration of PG. Table 2 shows the effect of PG on the value of D and K in the percutaneous DFS penetration, by computer simulation. The value of D in the percutaneous DFS penetration are significantly increased depending on the PG concentrations, but the value of K in the percutaneous DFS penetration are significantly decreased in the presence of PG, except for 1% PG system. The enhancing effect of PG on the percutaneous penetration of DFS was not proportional to the concentration of PG. We consider that the action site of PG may be mainly the intercellular lipid domains (intercel-

Table 1
Determination of diffusion coefficient (D) and partition coefficient (K) on the percutaneous penetration of tenoxicam by computer simulation

Treatment	D (cm^2/h, $\times 10^{-5}$)	K ($\times 10^{-3}$)
Control (no treatment)	4.654 ± 0.519	0.464 ± 0.519
1% PG	12.208 ± 3.019*	3.968 ± 1.640*
2% PG	16.376 ± 0.791***	4.129 ± 1.152**
3% PG	16.989 ± 1.373***	7.030 ± 0.543**
6% PG	23.376 ± 0.439***	7.636 ± 1.734**
10% PG	16.225 ± 4.239*	5.216 ± 1.618*

* Significantly different from control at $P < 0.05$.
** Significantly different from control at $P < 0.01$.
*** Significantly different from control at $P < 0.001$.

Table 2
Determination of diffusion coefficient (D) and partition coefficient (K) on the percutaneous penetration of diclofenac Na by computer simulation

Treatment	D (cm^2/h, $\times 10^{-5}$)	K ($\times 10^{-2}$)
Control (no treatment)	3.175 ± 0.443	1.368 ± 0.363
1% PG	5.825 ± 0.856**	4.013 ± 1.157**
2% PG	6.036 ± 0.766***	1.818 ± 0.296
3% PG	7.652 ± 2.016*	0.739 ± 0.102*
6% PG	11.005 ± 2.140**	0.625 ± 0.337*
10% PG	7.371 ± 0.263***	0.813 ± 0.165*

* Significantly different from control at $P<0.05$.
** Significantly different from control at $P<0.01$.
*** Significantly different from control at $P<0.001$.

lular route) in the SC, because the percutaneous penetration of TEX (water-insoluble drug) that may mainly pass through the intercellular lipid pathway in the SC is dependent on the concentration of PG. On the other hand, the effect of PG on the percutaneous DFS penetration may be increase only the, D value of DFS molecule. Further, we consider that the action site of PG may be mainly the intercellular lipid domains (intercellular route) in the SC, because the percutaneous penetration of TEX (water-insoluble drug) that may mainly passes through the intercellular lipid pathway in the SC is dependent on the concentration of PG, while in contrast the percutaneous penetration of DFS, which mainly passes through the polar pathway, is not dependent on the concentration of PG. Traditional permeability studies of full-thickness skin have implied that molecules permeated through the skin by either a polar or a nonpolar pathway, depending on the hydrophilicity or lipophilicity of the permeant and permeabilities of a number of permeants of varying solubility were studies using human cadaver skin (Knutson et al., 1985). The barrier effect of the SC against nonpolar solutes has been attributed to the interstitial lipids by a number of investigators. Michaels et al. (1975) attempted to correlate the barrier effects of the skin in terms of the composition of the pathways, as well as the microstructure, permeability, and selectivity of permeability of the particular pathways.

3.3. Accumulation of drugs in skin: in vitro study

The accumulation pattern of TEX did not have the same tendency as the penetration pattern, because the accumulation was not significantly increased with 1–3% PG then was increased with 6 and 10% PG (Fig. 3a), while the penetration enhancement showed parabolic relationship with PG concentrations. This phenomenon is by reason of increasing both D and K of TEX depending on the PG concentrations (Table 1). Further, we speculate that the action site of PG may be mainly the lipophilic site in the SC, PG efficiently enhanced the accumulation of TEX through the lipophilic pathway of the SC; however, PG enhanced DFS accumulation even more efficiently (2.5–5-folds increase as compared with the control, while increase in TEX accumulation ranged 1.5–2-folds increase as compared with the control, while increases in TEX accumulation ranged 1.5–2-folds, based on Fig. 3a and b). Fig. 3b shows the effects of PG on the accumulation of DFS in the dorsal skin of guinea pigs after 48 h, in vitro. The accumulation of DFS in skin caused by PG is inversely proportional to the PG concentration, because the K of DFS is inversely proportional to the PG concentrations (Table 2). Further, the D of DFS is slightly affected more than that of TEX (Tables 1 and 2). Furthermore, we speculate that the differences in the accumulation pattern of both drugs caused by PG may be due to differences in the accumulation site in skin of guinea pigs, which site are lipid-rich domains (TEX) or protein-rich domains (DFS). Consequently, we think that further studies are needed to analyze the mechanism of the drug accumulation in skin.

3.4. Effects of the PG concentration on the C–H bond stretching absorbance frequency shifts in the SC of guinea pigs, in vitro

The results presented in Fig. 4 compare the ATR-FTIR spectra in the C–H bond stretching

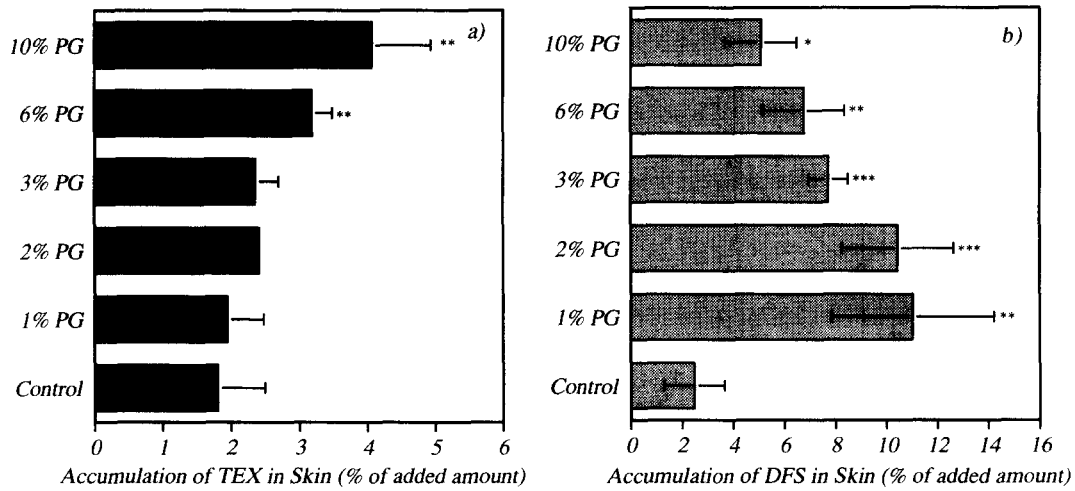

Fig. 3. (a) Effects of PG on the accumulation of TEX in skin of guinea pigs after 48 h, in vitro. ** Significantly different from control at $P < 0.01$. (b) Effects of PG on the accumulation of DFS in skin of guinea pigs after 48 h, in vitro. * Significantly different from control at $P < 0.05$. ** Significantly different from control at $P < 0.01$. *** Significantly different from control at $P < 0.001$.

region for an untreated sample (bottom trace) to the spectra of a sample treated with 3, 6, and 10% PG (top trace). Compared with the control, treatment of SC with PG results in a shift to a higher frequency and an absorbance broadening for both the C–H symmetric (near 2850 cm^{-1}) and the C–H asymmetric (near 2920 cm^{-1}) absorbances depending on PG concentrations. Frequency changes following frequent with various concentrations of PG in infrared band assignments for the SC of guinea pigs, in vitro, are summarized in Table 3. This result suggests that exogenously

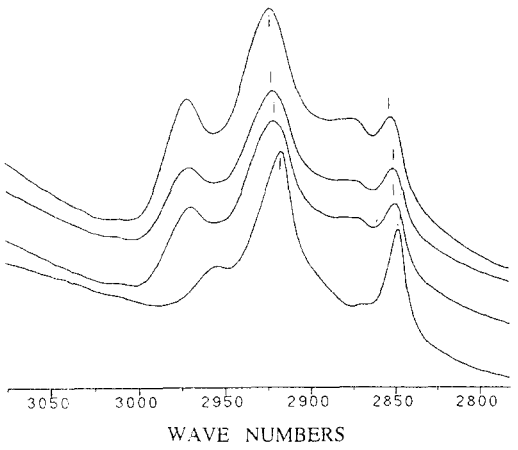

Fig. 4. The ATR-FTIR spectra of the stratum corneum of guinea pig dorsal skin in the C–H stretching region in the untreated stratum corneum (bottom trace), and stratum corneum treated with 3, 6, and 10% phosphatidylglycerol in propylene glycol (top trace). Both spectra were obtained at 25°C.

Table 3
Effects of PG on infrared band assignments for stratum corneum of guinea pigs, in vitro

Treatments of PG	C–H symmetric stretching Absorbance ($2850\ cm^{-1}$)	C–H asymmetric stretching Absorbance ($2920\ cm^{-1}$)
No treatment	2850.31 ± 0.00[a]	2919.73 ± 0.00
Propylene glycol (vehicle)	2850.31 ± 0.00	2919.73 ± 0.00
1% PG	2850.31 ± 0.00	2918.45 ± 1.11
2% PG	2851.28 ± 1.36	2922.63 ± 1.36
3% PG	2852.24 ± 0.00	2921.66 ± 0.00
6% PG	2852.88 ± 1.11	2923.59 ± 0.00
10% PG	2854.17 ± 0.00	2924.88 ± 1.11

[a] Value represents the average ± S.D. of three samples.

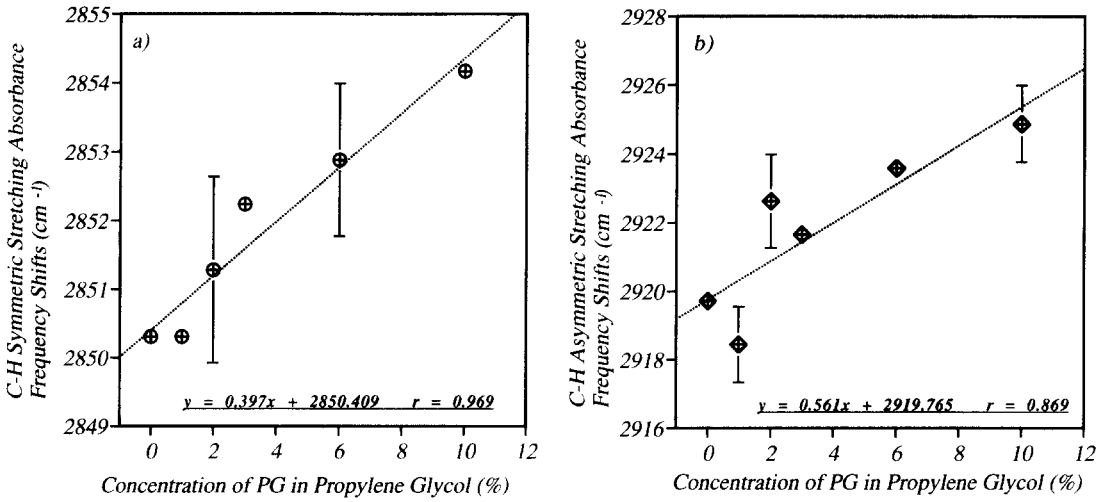

Fig. 5. (a) Effect of PG concentration on the C–H symmetric stretching absorbance frequency shifts in the stratum corneum of the guinea pig dorsal skin, in vitro. Data are the mean ± S.D. ($n = 3$). (b) Effect of PG concentration on the C–H asymmetric stretching absorbance frequency shifts in the stratum corneum of guinea pig dorsal skin, in vitro. Data are the mean ± S.D. ($n = 3$).

applied PG can disrupt the SC lipid structure. Fig. 5a shows the relationship between PG concentration and the C–H symmetric stretching absorbance frequency shifts in the SC of guinea pig dorsal skin, in vitro. The correlation coefficient obtained for the linear fit of the C–H symmetric stretching absorbance shifts versus the dose of PG, is high: $r = 0.969$. Fig. 5b shows the relationship between PG concentration and the C–H asymmetric stretching absorbance frequency shifts in the SC of guinea pig dorsal skin, in vitro. The correlation coefficient obtained for the linear fit of the C–H asymmetric stretching absorbance frequency shifts versus the dose of PG is relatively high: $r = 0.869$. Briefly, these results suggest that the SC's IR spectra exhibiting a sharp increase in bandwidth, and shifts of the C–H bond stretching absorbance depending on the PG concentrations are due to increased lipid fluidity in the SC lipids involving enhanced motional freedom of the hydrocarbon chains. Furthermore, these results indicate that PG may mainly more affect the C–H asymmetric stretching regions than the C–H symmetric stretching regions in the intercellular lipid domains (Slope in Fig. 5: C–H symmetric, region, $0.397x$; C–H asymmetric region, $0.561x$) The three spectral regions associated with C–H stretching, bending, and rocking vibrations substantiated the existence of solid to liquid phase transitions occurring in the hydrocarbon tails of the SC lipids. While the macroscopic studies associated increased permeabilities of lipophilic molecules through the SC with thermal transitions of the lipophilic pathways, the molecular origins were not identified. Intact SC and lipids extracted from the SC were further explored using FT-IR spectroscopy in order to elucidate the molecular origin of the increased permeability (Knutson et al., 1985).

3.5. Relationship between the C–H bond stretching absorbance frequency shifts and the percutaneous penetration of tenoxicam (TEX) and diclofenac Na (DFS) caused by PG, in vitro

Fig. 6a shows the relationship between the C–H symmetric stretching absorbance frequency shifts in the SC and the percutaneous TEX penetration caused by PG after 24 h and 48 h, in vitro. The correlation coefficient obtained for the appropriate linear fit is high: $r = 0.838$ (after 24 h) and $r = 0.825$ (after 48 h). Fig. 6b shows the correlation between the C–H asymmetric stretching absorbance frequency shifts in the SC and the percutaneous TEX penetration caused by PG after 24 h and 48 h, in vitro. The correlation

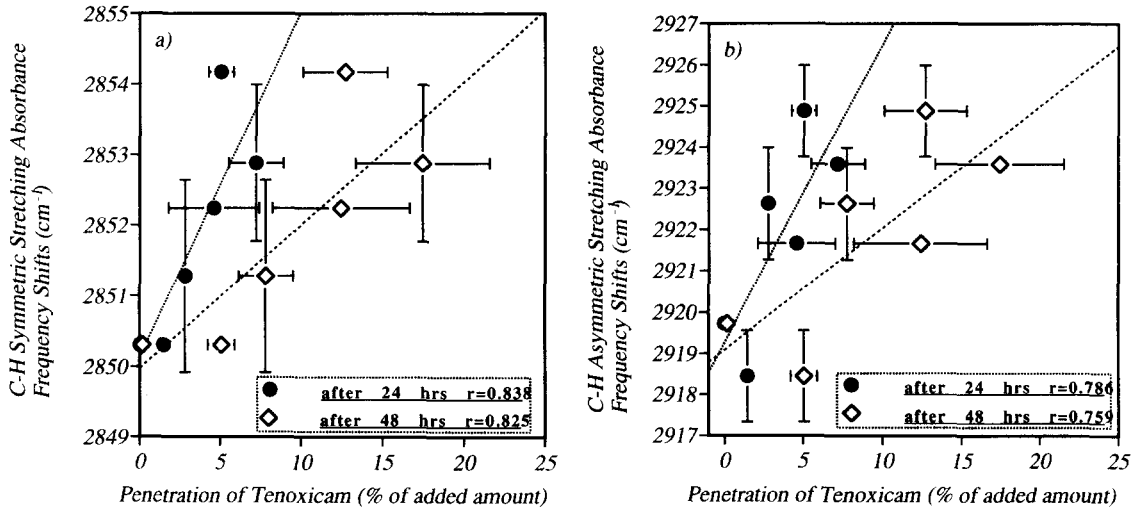

Fig. 6. (a) Relationship between the C–H symmetric stretching absorbance frequency shifts and the percutaneous penetration of TEX through the dorsal skin of guinea pigs after 24 h and 48 h, in vitro. Data are the mean ± S.D. ($n = 3$–4). Concentrations of PG are 0%, 1%, 2%, 3%, 6%, and 10%. (b) Relationship between the C–H asymmetric stretching absorbance frequency shifts and the percutaneous penetration of TEX through the dorsal skin of guinea pigs after 24 h and 48 h, in vitro. Data are the mean ± S.D. ($n = 3$–4). Concentrations of PG are 0%, 1%, 2%, 3%, 6%, and 10%.

coefficient obtained for the linear fit is relatively high: $r = 0.786$ (after 24 h) and $r = 0.759$ (after 48 h). These results suggest that the percutaneous penetration of TEX (water-insoluble drugs) caused by PG may be ultimately concerned with the lipid fluidity in the SC. Furthermore, we consider that the TEX molecule, which is water-insoluble, mainly passes through the intercellular lipid domains (non-polar pathway). Consequently, the significant parallel between the enhancement of the percutaneous TEX penetration and the measured SC lipid fluidity shifts caused by PG, suggests that the transdermal water-insoluble drug penetration may be ultimately related to the intercellular lipid lamellar structures of the SC. Fig. 7a shows the relationship between the C–H symmetric stretching absorbance frequency shifts in the SC and the percutaneous DFS penetration caused by PG after 24 h and 48 h, in vitro. The correlation coefficient obtained for the appropriate linear fit is low: $r = 0.455$ (after 24 h) and $r = 0.262$ (after 48 h). Fig. 7b shows the correlation between the C–H asymmetric stretching absorbance frequency shifts in the SC and the percutaneous DFS penetration caused by PG after 24 h and 48 h, in vitro. The correlation coefficient obtained for the linear fit is low: $r = 0.333$ (after 24 h) and $r = 0.131$ (after 48 h). These results suggest that the percutaneous penetration of DFS (water-soluble drugs) caused by PG may not be concerned with the lipid fluidity in the SC. Further, we consider that the DFS molecule, which is water-soluble, mainly passes through the intracellular protein domains (polar pathway). Consequently, the non-parallel between the enhancement of the percutaneous DFS penetration and the measured SC lipid fluidity shifts caused by PG, suggests that the transdermal water-soluble drug penetration may not be ultimately related to the intercellular lipid lamellar structures of the SC. Since lipophilic permeants were likely to partition into lipid matrices within the SC, the combined flux and FR-IR studies suggested that chemical enhancers' promotion of permeability results from increased fluidity of the lipids. Such studies have enabled investigators to probe the general nature of permeability pathways indirectly through knowledge of the structure of permeant. However, the molecular structure and organization of the pathways often remained unknown. The advent of instrumental techniques having enhanced sensitivity has allowed studying the molec-

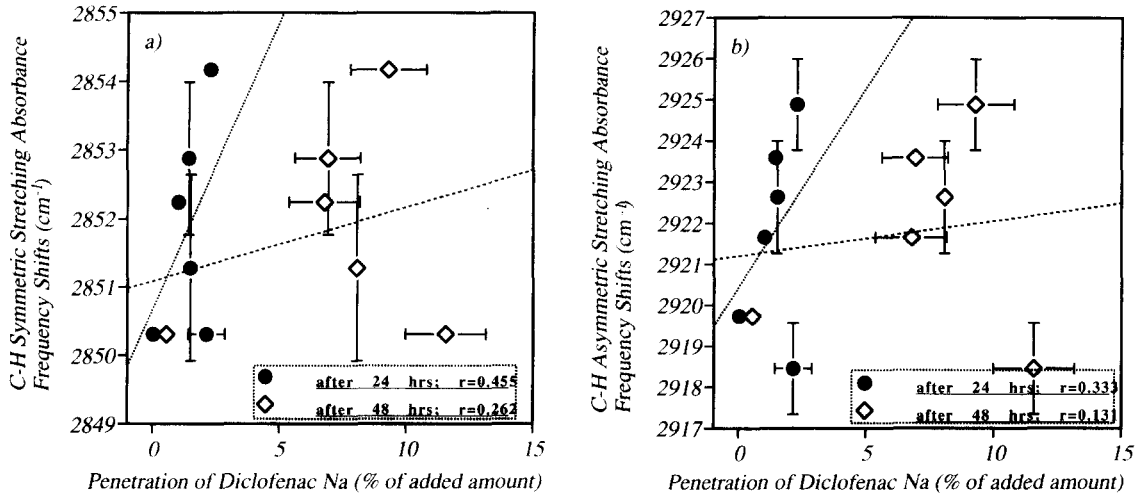

Fig. 7. (a) Relationship between the C–H symmetric stretching absorbance frequency shifts and the percutaneous penetration of DFS through the dorsal skin of guinea pigs after 24 h and 48 h, in vitro. Data are the mean ± S.D. ($n = 3$–4). Concentrations of PG are 0%, 1%, 2%, 3%, 6%, and 10%. (b) Relationship between the C–H asymmetric stretching absorbance frequency shifts and the percutaneous penetration of DFS through the dorsal skin of guinea pigs after 24 h and 48 h, in vitro. Data are the mean ± S.D. ($n = 3$–4). Concentrations of PG are 0%, 1%, 2%, 3%, 6%, and 10%.

ular mechanisms involved in SC barrier functions. In this study, we think that the enhancing mechanism of PG is due to fluidize the lipid hydrophobic matrices within the SC, resulting in decreased diffusional resistance to lipophilic permeants. The molecular structure and organization of the polar pathway in the SC for water-soluble drugs have been further explored by X-ray. X-ray diffraction and IR spectroscopy studies have also shown α to β conformational changes occurred in keratin and SC protein components with hydration or exposure to increased temperatures. Oertel (1977) has reported that pretreatment with dimethylsulfoxide resulted in the formation of β-sheet protein conformations in vitro in human SC. We consider that the mechanism of water-soluble drug penetration is very interesting and further needed to be studied.

3.6. Correlation between the C–H bond stretching absorbance frequency shifts and the accumulation of TEX in skin of guinea pigs caused by PG, in vitro

Fig. 8a shows the correlation between the C–H symmetric stretching absorbance frequency shifts and the accumulation of TEX in skin of guinea pigs caused by PG after 48 h, in vitro. The correlation coefficient obtained for the linear fit is remarkably high: $r = 0.958$. Fig. 8b shows the correlation between the C–H asymmetric stretching absorbance frequency shifts and the accumulation of TEX in skin of guinea pigs caused by PG after 48 h, in vitro. The correlation coefficient obtained for the linear fit is significantly high: $r = 0.902$. These results suggest that the accumulation of TEX in skin is more closely associated with the C–H bond stretching absorbance shifts caused by PG, than the percutaneous TEX penetration, in vitro (Fig. 5a, b). The higher-wavenumber shifts of the C–H asymmetric stretching vibration are associated with an increase in the number of 'gauche' conformers along the lipid hydrocarbon chains (Casal and Mantsch, 1984). It is well known that increases in width of bands and frequency of the C–H asymmetric stretching peak are due to transition in SC lipids involving enhanced motional freedom of the hydrocarbon chains (i.e. increased lipid fluidity) (Small, 1984). It was reported that the ATR-FTIR technique has indeed allowed the in vivo percutaneous penetration enhancement of model drugs to be monitored

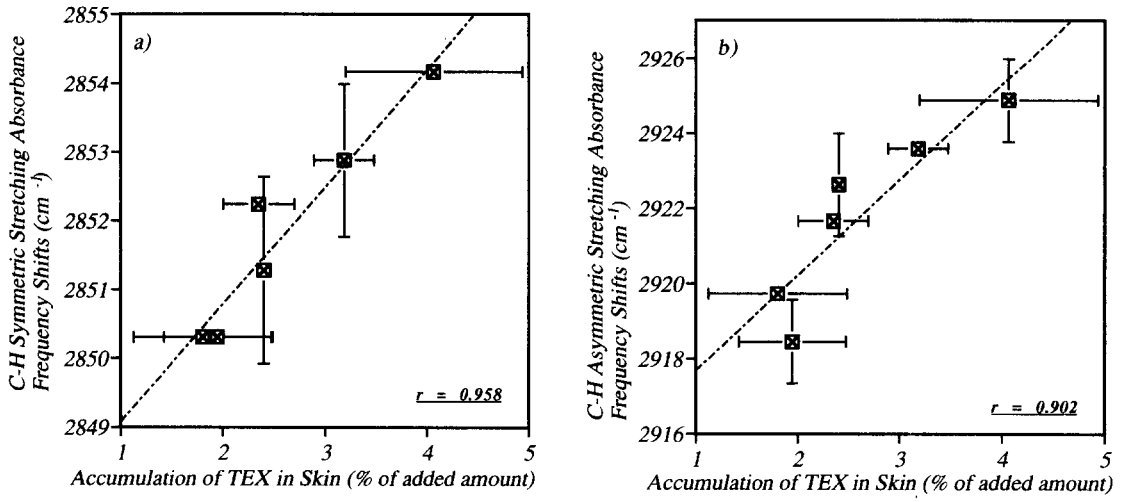

Fig. 8. (a) Relationship between the C–H symmetric stretching absorbance frequency shifts and the accumulation of TEX in the dorsal skin of guinea pigs after 48 h, in vitro. Data are the mean ± S.D. ($n = 3$–4). Concentrations of PG are 0%, 1%, 2%, 3%, 6%, and 10%. (b) Relationship between the C–H asymmetric stretching absorbance frequency shifts and the accumulation of TEX in the dorsal skin of guinea pigs after 48 h, in vitro. Data are the mean ± S.D. ($n = 3$–4). Concentrations of PG are 0%, 1%, 2%, 3%, 6%, and 10%.

(Mak et al., 1990). Overall, we suggest that this ATR-FTIR technique is applicable to in vitro percutaneous penetration of TEX and DFS caused by PG. Since SC lipids contain a large amount of free and esterified long chain fatty acids (Golden et al., 1987), we consider that exogenous PG, which has similar polarities, and which has the same saturated and unsaturated acyl chains in hydrophobic groups, may be easily partitioned into this domain. Saturated and *cis*-monounsaturated fatty acids have very different lipid packing properties, due to a 'kink' in the *cis*-alkenyl chain (Small, 1984). Additionally, the ability of *cis*-monounsaturated fatty acids disrupted the SC lipid structure and enhanced transdermal penetration can be explained by their 'kinked' nature. In this study, we consider that PG that has *cis*-monounsaturated acyl chains in its hydrophobic groups (mainly oleic acid) has a similarly 'kinked' nature. Further, we think that since PG also has *cis*-alkenyl chains, the incorporation of PG into the primarily saturated-like domain of the SC lipids causes increasing fluidity due to differences in lipid packing and disrupting the SC lipid structure. These results show that the maximum lipid fluidity is achieved for membrane containing fatty acid with 10% PG. We consider that maximum disruption of the SC barrier lipid may similarly result from incorporation of PG into the center of the lipid bilayers to disrupt the SC lipid structure and enhance the percutaneous penetration of TEX and DFS. Small reported that penetrant enhancers should have properties similar enough to the SC lipids to allow significant partitioning into this domain, yet dissimilar enough to maximally disrupt lipid packing (Small, 1984). The barrier to perrmeation in the SC was attributed not only to the interstitial lipids but also to their structures as ordered multilayers (Michaels et al., 1975). Thus, permeability studies suggest that lipophilic molecules permeate the SC through intercellular or interstitial lipid pathways. However, permeability studies only probe the barrier effect through the chemical and physical structures of the permeants, rather than examining the structure of the pathway itself. We consider that the structure of the pathway is important to precisely analyze the mechanism of the percutaneous drug penetration.

3.7. Localization of PG

Table 4 shows the recovery amount of PG after 48 h. PG was almost completely recovered from the donor side, which is in vehicles or maybe in shallow site in the stratum corneum. In a previous paper (Yokomizo and Sagitani, 1996a), the penetration-enhancing ability of phospholipids was almost proportional to their concentration. For example, treatment with 5% phospholipids was superior to treatment with 1% phospholipids in the percutaneous penetration of indomethacin, in vitro. In this study, PG was almost completely recovered from the donor side after, except for the 1% PG system. This result suggests that a high concentration of phospholipid molecules in vehicles contacting the SC of the skin surface is needed to strongly disrupt the lipid bilayers of the SC, and to strongly enhance the percutaneous penetration of TEX (PG concentrations up to 6%), except for DFS. It had been reported that most of liposomal lecithin was located in the horny layer (stratum corneum), and that there existed a steep concentration gradient from the skin surface to deeper horny layers (Wohlrab et al., 1989). Their results is almost in agreement with our results. Consequently, we consider that if a phospholipid's molecules are only in contact with the SC surface and are only slightly penetrated into the SC, they disrupt the barrier function of lipid bilayers in the SC, to enhance the percutaneous penetration of drugs. Overall, we consider that PG is almost not incorporated into the skin of guinea pigs, and as a result PG may be both safe and useful as a percutaneous penetration enhancer.

Table 4
Localization of PG after 48 h

Treatments	In skin (%)	In donor site (%)	
		Mean	S.D.
1% PG	N.D.	60.051	1.195
2% PG	N.D.	92.725	11.263
3% PG	N.D.	93.896	11.138
6% PG	N.D.	125.802	0.434
10% PG	N.D.	105.778	7.989

N.D., not detected in the HPLC system used.

4. Conclusion

(1) PG enhanced significantly the percutaneous penetration and accumulation of both the hydrophilic (DFS) and hydrophobic (TEX) drug, in vitro. (2) The enhancing mechanism of PG to TEX may be not only increasing the D and K of TEX but also increasing fluidity of the rout (intercellular lipid domain) for TEX, while that of PG to DFS may be increasing the D and K of DFS only. (3) Exogenously applied PG can increase efficiently the intercellular lipid fluidity in the SC; a shift to higher frequency and an absorbance broadening for the C–H bond region in the SC lipid, depending on the PG concentration (up to 10%). Further, PG mainly more affect C–H asymmetric stretching regions than C–H symmetric stretching regions in the intercellular lipid domain of the SC on the skin surface. (4) The striking parallel between the enhancement of the percutaneous penetration of TEX and the measured SC lipid fluidity shifts caused by PG, suggests that the transdermal water-insoluble drug penetration may be ultimately related to the SC lipid structure. (5) The non-parallel between the enhancement of the percutaneous penetration of DFS and the measured SC lipid fluidity shifts caused by PG, suggests that the mechanism of the transdermal water-soluble drug penetration may not be related to the SC lipid structure. (6) Since PG is almost not penetrated into the guinea pig skin, PG may be both safe and useful as a percutaneous penetration enhancer.

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