Drug Development and Industrial Pharmacy, 2009; 35(11): 1356–1363

informa
healthcare

ORIGINAL ARTICLE

Why does a hydrophilic drug permeate skin, although it is not soluble in white petrolatum?

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Abstract

Background: White petrolatum is broadly used as an ointment vehicle, although hydrophilic drugs cannot be easily dissolved in the vehicle. *Method*: The aim of this study was to evaluate the release and skin permeation profiles of a model hydrophilic agent, *N*1-[2-(4-guanidinophenyl)-1(S)-(*N*-methylcarbamoyl)ethyl]-*N*4-hydroxy-2(R)-iso-butyl-3(S)-(3-phenylpropyl)succinamide hydrochloride (FYK-1388b), from the ointment. *Results*: The release rate of FYK-1388b was very low; however, high skin permeation and skin content of the drug were found. We supposed that this was due to endogenous lipids or sebum, because white petrolatum had a high affinity to these lipids. To evaluate the effect of lipids on the enhanced release and skin permeation of FYK-1388b, 'preapplied white petrolatum' was made by applying the drug-free white petrolatum on the hairless rat skin for 6 hours. Then the drug ointment was prepared using the 'preapplied white petrolatum'. The release rate of FYK-1388b was markedly increased from the 'preapplied ointment' compared with the 'original ointment'. In addition, much higher skin permeation was also obtained using the 'preapplied ointment'. Separately, cholesteryl oleate, cholesterol, and ceramides were found in the 'preapplied white petrolatum'. *Conclusion*: Thus, these endogenous lipids on the skin surface may enhance the release and skin permeation of FYK-1388b from white petrolatum ointment.

Key words: *Ceramide; drug release; hydrophilic drug; ointment; sebum; skin permeation; white petrolatum*

Introduction

A variety of topical drug formulations are used, such as ointments, creams, lotions, liquids, and pastes[1–3]. Topical formulations are frequently utilized to treat atopic dermatitis and some are prescribed in hospitals. White petrolatum is used as an ointment vehicle for many drugs because of its low skin irritation. It is a noncrystal mineral ointment base. Physical properties of white petrolatum are density, 0.9 g/cm^3; boiling point, 302°C; melting point, 36–60°C; water solubility, not soluble; log *n*-octanol/water partition coefficient, 6. It is very stable against light and humidity. The composition of white petrolatum is expressed as C_nH_{2n+2}. It is a colloidal system of nonstraight-chain solid hydrocarbons, where most of the liquid hydrocarbons are held inside the micelle-like structure[4]. Several white petrolatum ointments containing lipophilic compounds, such as corticosteroid anti-inflammatory agents, have already been evaluated for their physicochemical properties and therapeutic effects[5–8]. The ointment vehicle, however, is highly hydrophobic, so that it is difficult to dissolve and entrap hydrophilic potent drugs. Thus, few studies have been performed on the release and skin permeation of hydrophilic drugs from white petrolatum ointments.

To evaluate the potency of white petrolatum as an ointment base for hydrophilic drugs, we selected a new anti-atopic drug, *N*1-[2-(4-guanidinophenyl)-1(S)-(*N*-methylcarbamoyl)ethyl]-*N*4-hydroxy-2(R)-iso-butyl-3(S)-(3-phenylpropyl)succinamide hydrochloride (FYK-1388b) (Figure 1), which inhibits matrix metalloproteinase to affect atopic dermatitis, for a test model hydrophilic compound. White petrolatum ointment containing FYK-1388b was prepared to evaluate its release and skin permeation

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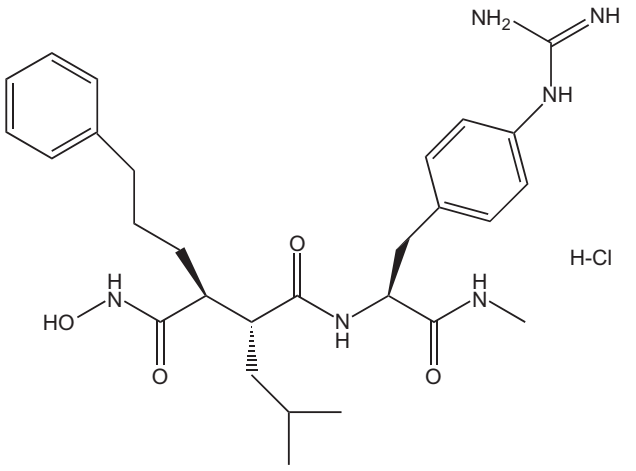
(Received 17 Nov 2008; accepted 1 Apr 2009)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.3109/03639040902939205



Molecular weight	561.13
n-Octanol–water partition coefficient	(pH 2) 0.01 less or equal (pH 4) 0.02 (pH 6–10) 0.1–0.13
Solubility	Water: ≥20 mg/mL Ethanol: ≥10 mg/mL
pK_a	About 9

Figure 1. Chemical structure and physicochemical properties of FYK-1388b.

properties. We then used the obtained results to evaluate whether this hydrophilic drug can be released from the ointment and permeate the skin. First, an aqueous solution of FYK-1388b was applied to excised hairless rat skin to measure the in vitro skin permeability of the drug. Second, the release and skin permeation profiles of FYK-1388b were determined from white petrolatum ointment. In addition, the effect of endogenous lipids (containing sebum, ceramide, and so on) on the release and skin permeation of the hydrophilic drug was evaluated. Finally, the release and skin permeation mechanism of the drug from white petrolatum ointment were discussed using the obtained results. In particular, it was discussed whether white petrolatum ointment containing hydrophilic drugs could be put to practical use.

Materials and methods

Materials and experimental animals

FYK-1388b was a gift from Daiichi Fine Chemical Co., Ltd. (Takaoka, Toyama, Japan). White petrolatum was obtained from Kosakai Pharmaceutical Co., Ltd. (Tokyo, Japan). Nonhydroxy fatty acid ceramide (NHFC) was purchased from Sigma-Aldrich Chemical Co., Ltd. (St. Louis, MO, USA). Triolein, cholesteryl oleate, and oleic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals and solvents were of reagent grade or high-performance liquid chromatography (HPLC) grade and were used without further purification.

Male hairless rats (WBM/ILA-Ht; 7–8 weeks old; body weight: 220–270 g) were from the Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animals (Fukaya, Saitama, Japan).

Preparation of FYK-1388b solution and white petrolatum ointment

FYK-1388b solution was prepared at a concentration of 10 mg/mL (1.0%) with distilled water.

FYK-1388b white petrolatum ointment at concentrations of 10 mg FYK-1388b/g and 30 mg FYK-1388b/g (1.0% and 3.0%) was prepared by thoroughly mixing with white petrolatum to prepare 'original ointment'. To evaluate the effect of endogenous lipids extracted from hairless rat skin on drug release, white petrolatum base was pretreated over 6 hours on the full-thickness skin (application of about 300 mg base on the 0.95 cm^2 skin) and collected. The pretreated white petrolatum was used to prepare 1.0% FYK-1388b 'preapplied ointment'. The mixing of FYK-1388b to the 'original' and 'preapplied' white petrolatum was done just before the experiments.

Release experiments

The test ointment (100 or 300 mg) was placed in a disk-shaped cell cap with an effective diffusion diameter of 1.6 cm and thickness of 2.5 mm. The cell cap was applied to the diffusion cell with an effective diffusion area of 0.95 cm^2 using silicone rubber, as shown in Figure 2a (no skin was sandwiched between the cells in the release experiment), to measure FYK-1388b release from the ointments. The receiver cell was filled with 3.0 mL of pH 7.4 phosphate-buffered saline (PBS). Aliquots (500 μL) were sampled at 5, 10, 15, 30, 60, 90, 120, 180, and 240 minutes to consecutively determine drug release from the ointments to PBS solution using HPLC.

In vitro skin permeation experiments

Male hairless rats were fixed on their back after anesthesia by i.p. injection of sodium pentobarbital, and hair on the abdomen was shaved using an electric shaver. In the skin permeation experiment using FYK-1388b solution, the excised skin (full-thickness skin or stripped skin) was set in a Franz-type diffusion cell with an effective permeation area of 3.14 cm^2, with the water jacket on the received cell connected to a water bath at 32°C (Figure 2b). Stripped skin was prepared by 20 consecutive tape-strippings of the stratum corneum from hairless rats[9]. The test solution (1.0% FYK-1388b, 2.0 mL) was applied to the epidermal side, whereas PBS

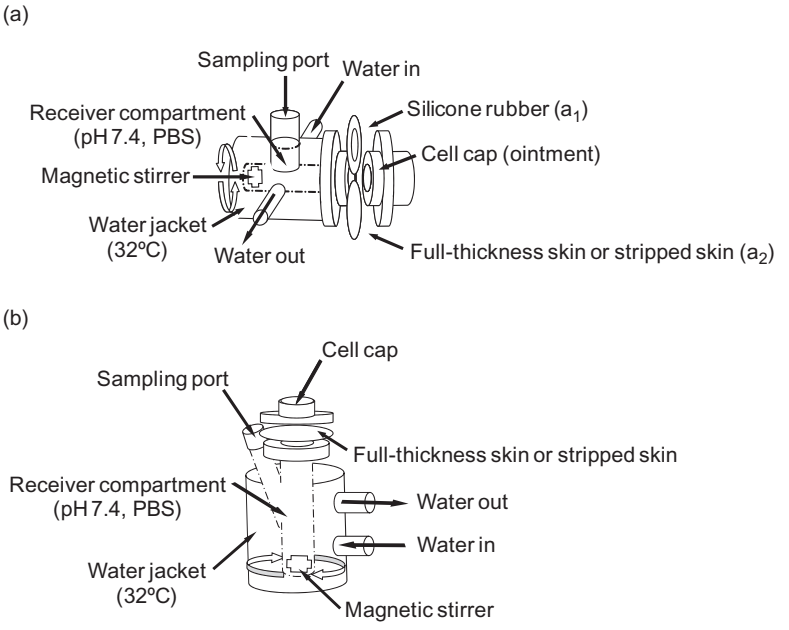


Figure 2. Experimental setup for release and in vitro skin permeation study using horizontal diffusion cell (a) and Franz-type (vertical) diffusion cell (b). Silicone rubber was used for the release study (a_1), whereas full-thickness skin or stripped skin was applied for the in vitro skin permeation study (a_2).

(16–18 mL) was applied to the dermal side. The receiver compartment was agitated using a magnetic stirrer and a stirrer bar. At predetermined times, a 500-μL aliquot was withdrawn from the receiver solution and the same volume of fresh PBS was added to keep the volume constant. In the permeation experiment using stripped skin, samples were taken every 1 hour until 8 hours. In this experiment, Franz-type cell was used, instead of the horizontal cell as shown in Figure 2a, because of its convenience. Skin permeation profile using Franz-type cell shown in Figure 2b must be equivalent to that using the cell shown in Figure 2a.

In the skin permeation experiments using white petrolatum ointment, each test ointment (~300 mg) was applied to the epidermal side of excised abdominal skin set in a diffusion cell with an effective permeation area of 0.95 cm^2, with the water jacket connected to a water bath at 32°C, whereas PBS (3.0 mL) was applied to the dermal side (Figure 2a). The receiver compartment was agitated as above. At predetermined times, a 500-μL aliquot was withdrawn from the receiver solution and the same volume of fresh PBS was added. Samples were taken every 1 hour until 6 hours for the experiments using stripped skin, whereas sampling times were 2, 4, 6, 8, 10, 12, and 24 hours for full-thickness skin. The amount of drug permeating the skin was determined using HPLC.

The amount of drug retained in the skin after the permeation experiment was also determined as follows. The excised abdominal skin was removed, and the ointment applied to the skin surface was cleaned off. The obtained skin sample was kept at −20°C until analysis. The frozen skin was finely cut and homogenized with 2.5 mL of PBS and the same volume of methyl 4-hydroxybenzoic acid as an internal standard (10 μg/mL in acetonitrile) on ice. The supernatant of this homogenate was injected into HPLC.

Determination of FYK-1388b

Each 200-μL sample from the release experiment and the in vitro skin permeation experiment was mixed with the same volume of internal standard (10 μg/mL of methyl 4-hydroxybenzoic acid in acetonitrile), and an aliquot (20 μL) was injected into HPLC. No significant metabolites of FYK-1388b were found on the HPLC chart of the sample.

The HPLC system consisted of a pump (LC-10AS; Shimadzu, Kyoto, Japan), a column (LiChrospher® 100 RP-18 endcapped, 4.0 × 250 mm; Merck KGaA, Darmstadt, Germany), a column oven (CTO-6A; Shimadzu, Kyoto, Japan) at 40°C, an auto-injector (SIL-10A; Shimadzu, Kyoto, Japan), a UV detector (SPD-10A; Shimadzu, Kyoto, Japan), and an integrator (C-R5A; Shimadzu, Kyoto, Japan). The mobile phase was acetonitrile : distilled water : 10% trifluoroacetic acid (25:74:1) and the flow rate was 1.0 mL/min for samples from the release experiment and in vitro skin permeation experiment, respectively. Detection was performed at UV 233 nm. This determination method has already been validated by KO at Daiichi Fine Chemical Co., Ltd. (Takaoka, Toyama, Japan).

Identification and semiquantitative determination of biological constitutes in white petrolatum preapplied to full-thickness skin

Thin-layer chromatography (TLC) analysis was performed to check for endogenous lipids in white petrolatum

preapplied to full-thickness skin as follows. The white petrolatum sample preapplied to dorsal full-thickness skin in hairless rats (application area: 48 cm^2, ~0.8 g) was dissolved in hexane. The dissolved hexane was passed through a solid-phase column, and endogenous lipids were extracted by chloroform:methanol (2:1) solution in a solid-phase column. The extracted chloroform : methanol (2:1) solution was concentrated under N_2 purge. The residue was reconstituted to add chloroform:methanol (2:1) solution (1 mL). These samples (10 µL) were applied to a TLC plate and developed using *n*-hexane:diethyl ethane:methanol (60:25:15) for about 20 minutes[10]. Detection was performed by placing 10% copper sulfate in 23% ethanol and 8% phosphoric acid and heating at 105°C for 10 minutes[10] and analyzed semiquantitatively by densitometer (Scion Image, Frederick, MD, USA).

A control sample for TLC analysis was used, which consisted of 1.0% NHFC, 1.0% cholesterol, and 1.0% synthetic sebum in chloroform : methanol (2:1) solution. The synthetic sebum consisted of 50% triolein, 30% cholesteryl oleate, and 20% oleic acid.

Analysis of statistics

Each data point is shown as the mean ± SE of three to seven experiments. Statistical analysis was performed by analysis of variance (ANOVA) with nonrepeated measures, and $P<0.05$ was assumed to be significant.

Theoretical analysis

The cumulative amount of drug released from ointment vehicle, Q_r, per unit area is expressed as a function of time, t, as follows[1,11]:

$$Q_r = \sqrt{(2C_0 - C_s)C_s D_v t} = A\sqrt{t}, \qquad (1)$$

where C_0 and C_s are the initial concentration and solubility of the drug in the vehicle, respectively, D_v shows the diffusion coefficient of the drug in the ointment vehicle, and A is $\sqrt{(2C_0 - C_s)C_s D_v}$. When a solid or crystal drug is present on the ointment surface or the release surface is increased with a passage of time by the ointment swelling, initial burst release may be observed because of burst dissolution of the solid/crystal drug or swelling of ointment base itself. Under the assumption that the initial burst release and the following release rate obeyed the first-order kinetics and Fickian diffusion, respectively, them Equation (1) becomes

$$Q_r = B(1 - e^{-kt}) \text{ at } 0 \le t \le t_0 \qquad (2)$$

$$Q_r = A\sqrt{t} + C \qquad \text{at } t_0 \le t < \infty, \qquad (3)$$

where B, C, and k are constants. A, B, C, and k were obtained by calculation from Equations (2) and (3) using least-squares method. The parameter t_0 can be obtained by a relation of Equation (2)=Equation (3) using a calculation software, Maple™ ver. 11 (Maplesoft, Waterloo, ON, Canada).

Results and discussion

In vitro skin permeation of FYK-1388b from aqueous solution

First, the in vitro skin permeation of FYK-1388b was determined from its aqueous solution. Figure 3a and b shows the cumulative amount of FYK-1388b that permeated through excised hairless rat skin (full-thickness skin and stripped skin) over time after application of 1.0% FYK-1388b in distilled water. Full-thickness skin

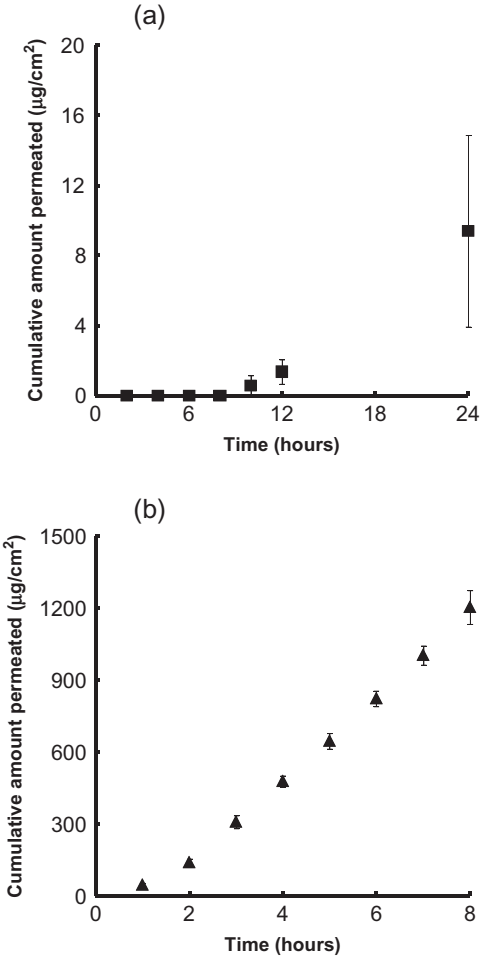


Figure 3. Cumulative amount of FYK-1388b permeating full-thickness skin from 1.0% aqueous solution (■) (a) and stripped skin from 1.0% aqueous solution (▲) (b). Each value shows the mean±SE (n=3 or 4).

Table 1. Amount of FYK-1388b retained in stripped skin and full-thickness skin after application of a solution or white petrolatum ointment with or without preapplication to full-thickness skin.

Vehicle	Full-thickness skin (μg/cm^2)	Stripped skin (μg/cm^2)
Aqueous solution	61.5±7.59	652±67.1
Original white petrolatum (control)	0.96±0.28	7.86±1.00
Preapplied white petrolatum	4.13±0.47*	9.56±0.76

Each value shows the means±SE (n=3 or 4). *Significant at P<0.01 when compared with control. Statistical analysis was performed by ANOVA with nonrepeated measures.

permeation was 9.39 μg/cm^2 over 24 hours, and stripped skin permeation was 1206 μg/cm^2 over 8 hours. The skin level of FYK-1388b is summarized in Table 1. The skin level after application to stripped skin (652 μg/cm^2 at 8 hours) was about 10 times higher than that of full-thickness skin (61.5 μg/cm^2 at 24 hours), suggesting that the stratum corneum had a role as the primary barrier against the skin permeation of FYK-1388b.

Evaluation of in vitro release and skin permeation of FYK-1388b from white petrolatum ointment

Shah et al.[12] and Shah[13] suggested that the in vitro release property of drugs from topical formulations can be used to validate the bioequivalence of generic formulations against the original. We prepared white petrolatum ointment containing FYK-1388b and performed a release experiment as well as an in vitro skin permeation experiment. FYK-1388b did not dissolve in the white petrolatum ointment. Crystals of the drug were found in the ointment by a light microscope.

In vitro release of FYK-1388b from white petrolatum ointment was determined using 100 and 300 mg of FYK-1388b ointments at a concentration of 1.0% or 3.0%. The obtained results are shown in Figure 4. Release of FYK-1388b was also determined from hydrophilic ointment and simple ointment for comparison. The results are shown in Figure 5. The drug release from simple ointment obeyed Higuchi's theory, whereas the drug release from white petrolatum and hydrophilic ointment simply did not obey the theory. The reason for the burst release of FYK-1388b from white petrolatum (Figure 4b) may be different from that of hydrophilic ointment (Figure 5, ■). The burst release for the hydrophilic ointment must be swelling of the ointment base by the receiver water, whereas that for white petrolatum is probably because of direct dissolution of FYK-1388b solid on the surface of ointment. The release surface of the hydrophilic ointment became convex after the initial release experiment. In case of white petrolatum, the drug crystals were found on the ointment surface by light microscope observation. Then the release profiles

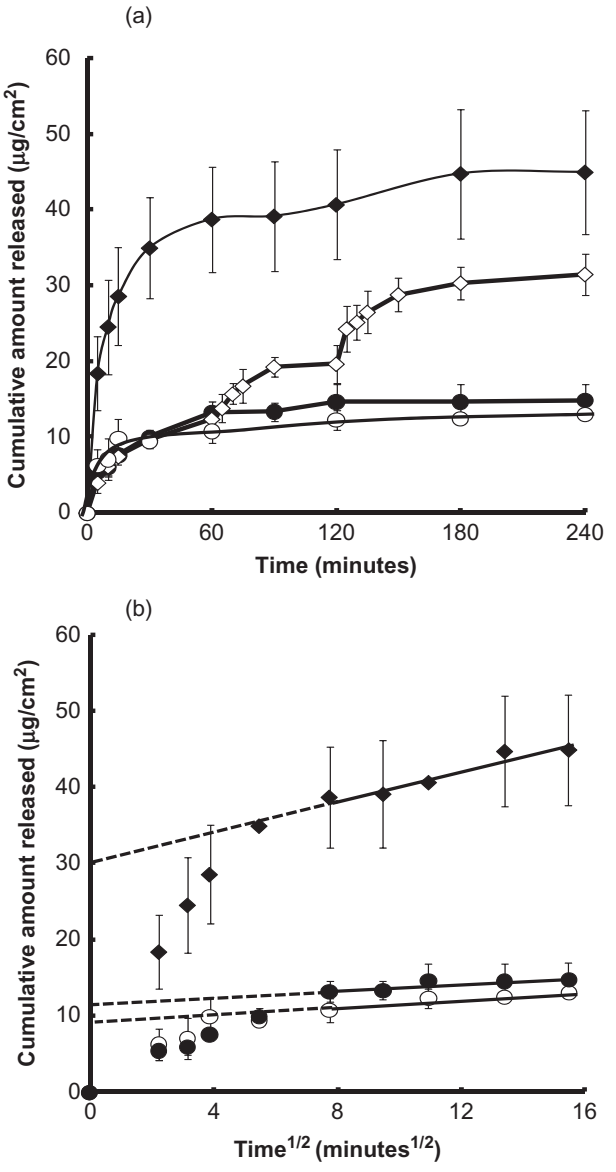


Figure 4. (a) Amount of FYK-1388b released from 100 mg of 1.0% FYK-1388b white petrolatum ointment (●), 300 mg of 1.0% FYK-1388b white petrolatum ointment (○) and with re-kneading the ointment at 60 and 120 minutes (✧), and 300 mg of 3.0% FYK-1388b white petrolatum ointment (♦). (b) Higuchi plot of FYK-1388b release from FYK-1388b white petrolatum ointment using the same data as shown in Figure 4a. (The same symbols were used as in Figure 4a.) Solid and dashed lines are calculated by the least-squares method. Each value shows the mean ± SE (n=3–7).

were characterized using parameter A, B, C, and k (Table 2), as explained in Equations (1)–(3).

Linear line on the cumulative amount versus square root of time curve in Figure 4b was observed from 40 to 80 minutes after the beginning of the release experiment. Although the applied amount (100 or 300 mg ointment; ○ and ● in Figure 4) was different [FYK-1388b concentration was the same (1.0%) and the area of the

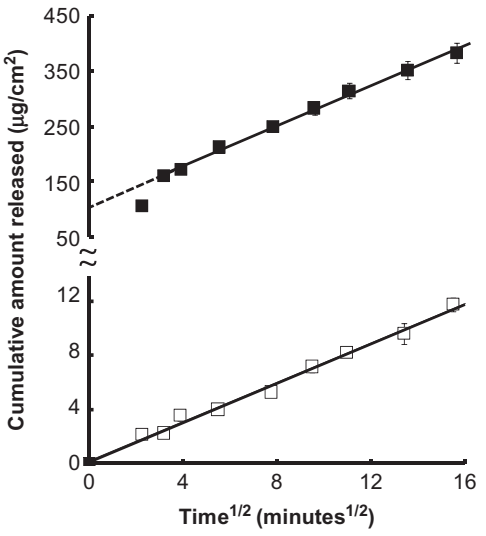


Figure 5. Higuchi plot of FYK-1388b release from 300 mg of 1.0% FYK-1388b simple ointment (□) and hydrophilic ointment (■). Solid and dashed lines are calculated by the least-squares method. Each value shows the mean ± SE (n=4–8).

released surface was the same], the cumulative amount of FYK-1388b released from 300 mg ointment was similar to that from 100 mg ointment. The index of burst release, C, in Equation (3), was calculated to be 11.7 and 8.88 μg/cm^2 and the slope of the square root plot, A, after t_0 was about 0.21 and 0.27 μg/cm^2/min$^{1/2}$ for 100 and 300 mg ointments, respectively (Table 2). Next, to evaluate the effect of drug concentration on FYK-1388b release, 3.0% FYK-1388b ointment was evaluated as well as 1.0% FYK-1388b. Initial burst was also observed for 3.0% ointment (♦ in Figure 4), but the C-value (26.4 μg/cm^2) was severalfold that for 1.0% ointment. In addition, the A-value (1.30 μg/cm^2/min$^{1/2}$) was also much higher than that for 1.0% ointment (Table 2). C_0 was much higher than C_s in the present FYK-1388b ointments, and the slope, A, is approximately expressed by $\sqrt{2C_0C_sD_v}$. As C_s and D_v are the same for 1.0% and 3.0% ointments, the difference in the slope between 1.0% and 3.0% ointments must be $\sqrt{3}$. In addition, the difference of the observed C-value between 1.0% and 3.0% ointment must be 3.0. The observed A- and C-values were a little different from these theoretical values but may be within the experimental error.

Further release experiments were performed by re-kneading the white petrolatum ointment 60 and 120 minutes after starting the experiment, where the ointment was taken from the release surface, kneaded, and applied again. As a result, an initial burst release was again observed from 60 to 120 minutes (✧ in Figure 4a). FYK-1388b was repeatedly released from the ointment surface immediately after it was kneaded and mixed. This is probably due to the dissolution of FYK-1388b crystals or solid moving from the inside to the release surface of the ointment.

These results suggest that the diffusion rate of FYK-1388b in white petrolatum ointment must be very low and that the highest fraction for drug release was dependent on the amount of solid or crystal drug at the ointment surface but not on the amount of ointment.

Next, a skin permeation experiment of FYK-1388b was performed using white petrolatum ointment (Figure 6). No permeation of FYK-1388b was observed through full-thickness skin, but it permeated stratum corneum-stripped skin (Figure 6, ❍).

Generally, therapeutic drugs in the formulation must be diffused and released through and from formulation vehicles, and then distributed, penetrating into and permeating the skin, to induce pharmacological effects by topical formulation. Although the diffusion rate of FYK-1388b in white petrolatum was very low, ointments containing many drugs are practically used for patients with the expectation of high pharmacological effects. These pharmacological effects of topical formulations are usually related to the skin concentration rather than the skin permeation of drugs. Table 1 also shows the skin concentration of FYK-1388b after topical application of 1.0% FYK-1388b white petrolatum ointment. FYK-1388b was detected in the skin despite no skin permeation of the drug through full-thickness skin, suggesting effective diffusion of the drug into the skin. Why does FYK-1388b permeate skin from white petrolatum ointment, although the drug is not soluble in the ointment?

Table 2. Release parameters of FYK-1388b from white petrolatum ointment.

	A[a] (μg/cm^2/min$^{1/2}$)	B (μg/cm^2)	C (μg/cm^2)	k (min^{-1})	t_0 (min)	r^2
Original white petrolatum						
1% FYK-1338b 100 mg	0.21	13.7	11.7	0.06	77.5	0.84
1% FYK-1338b 300 mg	0.27	10.9	8.88	0.13	55.6	0.92
3% FYK-1338b 300 mg	1.30	34.7	26.4	0.13	37.7	0.92
Preapplied white petrolatum						
1% FYK-1338b 300 mg	0.55	19.6	16.0	0.17	41.0	0.85

[a] $A=\sqrt{(2C_0-C_s)C_sD_v}$.

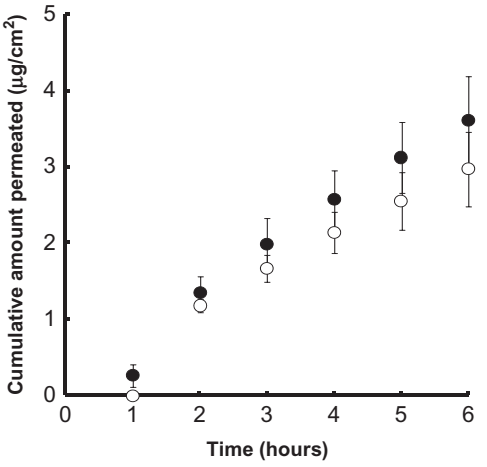


Figure 6. Cumulative amount of FYK-1388b permeating stripped skin from original white petrolatum ointment (○) and preapplied white petrolatum ointment (●) at a concentration of 1.0% FYK-1388b. Each value shows the mean ± SE (n=3 or 4).

Identification of endogenous skin lipids in 'preapplied white petrolatum'

The unexpectedly high skin concentration of FYK-1388b after application of white petrolatum ointment may be due to an occlusion effect by the applied white petrolatum itself and an increase in drug solubility in the ointment by endogenous lipids on and in the skin surface and stratum corneum. It was recently reported that petrolatum applied on skin surface influenced the lipid amount in the stratum corneum[14]. Then, we identified endogenous lipids in white petrolatum preapplied to full-thickness skin in hairless rats using TLC. The obtained results, shown in Figure 7, suggest that skin lipids, such as cholesteryl oleate, cholesterol, and ceramide, were extracted from the skin into the vehicle. The semiquantitative determination of endogenous lipids by a densitometer showed that 4.41 mg cholesteryl oleate, 0.52 mg cholesterol, and 0.22 mg NHFC were extracted from 1.0 g white petrolatum that was applied on the rat skin for 6 hours.

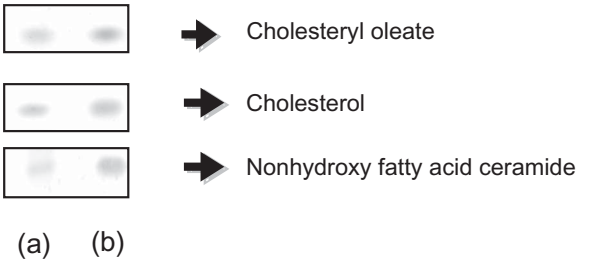


Figure 7. Thin-layer chromatogram of endogenous lipids extracted from preapplied white petrolatum ointment (a) and mixed solution of 1.0% synthetic sebum, 1.0% cholesterol, and 1.0% nonhydroxy fatty acid ceramide (b).

The solubility parameters of these endogenous lipids are 9.55 $(cal/cm^3)^{1/2}$ for cholesterol[15], 7.24 $(cal/cm^3)^{1/2}$ for cholesteryl oleate[15], 9.44 $(cal/cm^3)^{1/2}$ for NHFC[16], and 10.1 $(cal/cm^3)^{1/2}$ for ceramide II[16], similar to that of white petrolatum [7.33 $(cal/cm^3)^{1/2}$][15]. Thus, the extraction of such lipids to white petrolatum ointment is not an unlikely phenomenon. Additionally, the polar character of cholesterol is higher than that of white petrolatum and cholesterol has an emulsification effect; thus, cholesterol may contribute to the solubilization of hydrophilic drugs such as FYK-1388b.

Drug release and skin permeation from endogenous lipids-entrapped white petrolatum ointment

The effect of endogenous lipids on the release and skin permeation of FYK-1388b was evaluated. FYK-1388b ointment (1.0%) was prepared with 'preapplied white petrolatum', and drug release and skin permeation experiments were carried out. Figures 6 and 8 show the effect of 'preapplied white petrolatum ointment' on the skin permeation and drug release profiles, respectively. Stripped skin permeation from the 'preapplied ointment' (Figure 6, ●) was a little higher than that from the 'original ointment'. As shown in Figure 8 and Table 2, the release profile of FYK-1388b from 'preapplied white petrolatum ointment' was twice as high as that from the 'original (nonapplied) ointment'. In addition, slope of the square root plot, A, in the 'preapplied white petrolatum ointment' showed 2.0 times higher value compared with the 'original ointment'.

Interestingly, the skin level after application of 1.0% FYK-1388b in 'preapplied white petrolatum ointment' to stripped skin was the same as that of the 'original ointment', but the skin level after application of 1.0% FYK-1388b in 'preapplied white petrolatum ointment'

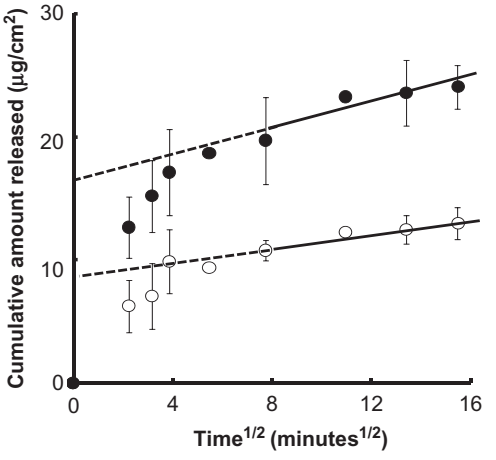


Figure 8. Higuchi plot of FYK-1388b release from white petrolatum (○) and preapplied white petrolatum ointment (●) at a concentration of 1.0% FYK-1388b. Solid and dashed lines are calculated by the least-squares method. Each value shows the mean ± SE (n=3 or 4).

to full-thickness skin was 4.3 times higher than that of the 'original ointment' (Table 1), suggesting that endogenous lipids entrapped in white petrolatum ointment affected the release and skin distribution of a hydrophilic drug, FYK-1388b, because of increased solubility (C_s) and diffusion coefficient (D) to FYK-1388b in hydrophobic white petrolatum.

To further ensure the effect of lipids on the release and skin permeation, 10% artificial sebum and 0.1% NHFC in white petrolatum ointment were evaluated. FYK-1388b release from 10% artificial sebum ointment (13.8 μg/cm^2 over 4 hours) was similar to that from 'original ointment', whereas that from 1.0% NHFC ointment (20.3 μg/cm^2 over 4 hours) was about 1.5 times to that from original one. No permeation of FYK-1388b from the lipid-containing ointment was found, and it was the same as the 'original ointment'. The drug permeation through the stripped skin from 10% artificial sebum in the ointment (6.02 μg/cm^2 over 6 hours) was twice, but that from 1.0% NHFC (2.56 μg/cm^2 over 6 hours) was similar to the 'original ointment'. Interestingly, a higher skin concentration was observed after application of 10% artificial sebum or 1.0% NHFC ointment on the full-thickness skin and the stripped skin (13.4 or 1.9 μg/cm^2 at 24 hours and 22.5 or 12.4 μg/cm^2 at 6 hours, respectively). These results suggest the effect of endogenous lipids extracted from the skin surface on the enhanced release and skin permeation of FYK-1388b. In addition, it is well known that the addition of wool fat to white petrolatum increases the affinity of hydrophilic drugs to white petrolatum ointment. Addition of water at a concentration of 1.0% and wool fat at a concentration of 10.0% to 1.0% FYK-1388b ointment slightly decreased and increased threefold the release amount (7.9 and 24.6 μg/cm^2 over 4 hours, respectively). Thus, endogenous lipids such as cholesteryl oleate, cholesterol, and ceramides show a similar effect as in wool fat. The decreased release by water may be because of dissolution of solid drug by water in the ointment. These endogenous lipids may change the barrier function of skin and probably increase the skin level of FYK-1388b. We have reported a similar effect of endogenous lipids on the solubility of lidocaine in pressure-sensitive adhesive tape formulations[17].

Further experiments are necessary to correctly identify the detailed mechanism, especially in atopic patients.

Conclusion

Although the in vitro release of FYK-1388b from white petrolatum ointment was low, ointments can be topically administered to evaluate the drug concentration in the skin. The reason for the skin permeation of FYK-1388b, a hydrophilic drug, from highly hydrophobic white petrolatum ointment may be related to the improved distribution of FYK-1388b from the ointment base to skin by the extraction of skin lipids.

Declaration of interest: The authors report no conflicts of interest.

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