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Analysis of *in vitro* skin permeation of 22-oxacalcitriol from ointments based on a two- or three-layer diffusion model considering diffusivity in a vehicle

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Abstract

In the present study, *in vitro* rat skin permeation of 22-oxacalcitriol (OCT) from ointments having differing compositions was determined and discussed based on a diffusion model. Diffusion coefficients of OCT in two ointments, one containing 3% (w/w) medium chain triglyceride (MCT) (3MO) and the other 30% (w/w) MCT (30MO), were determined using a modified membraneless method resulting in values of 0.89×10^{-4} and 1.87×10^{-4} cm^2/h, respectively. At 24 h after application with 3MO, 7% of the applied OCT dose permeated through full-thickness skin and 22% remained in the ointment, whereas with 30MO, 2% of the applied dose permeated through full-thickness skin and 65% remained in the ointment. The diffusion coefficient of OCT in 3MO was lower than 30MO but the cumulative amount of OCT permeated was higher. From analysis of skin permeation of OCT based on a diffusion model considering diffusivity in an ointment, the partition coefficient of OCT from the ointment to stratum corneum ($K_{SC/V}$) was calculated to be five-fold higher with 3MO than with 30MO. Our simulation study based on a diffusion model suggests that the diffusion coefficients of OCT in both ointments were high enough to have no affect on the skin permeation of OCT in the present case and that the difference in the skin permeations of the ointments was mainly caused by a difference in $K_{SC/V}$.

Keywords: Skin permeation; Formulation; Diffusion model; Partition; *In vitro* rat skin

1. Introduction

In the development of a drug for topical use, the formulation must be carefully designed to achieve good therapeutic efficacy. Investigators have evaluated the relationship between skin permeation of drugs and topical formulations such as gels, creams, and ointments. The *in vivo* skin stripping method (Shah et al., 1998; Weigmann et al., 1999; Pershing et al., 2002) and *in vitro* skin permeation experiments are useful for comparing the percutaneous absorption kinetics of different formulations. Permeation parameters such as diffusivity in a formulation and partitioning from formulation to skin are easily altered by the composition of the formulation (Díez-Sales et al., 2005).

Thus, partitioning from a formulation to skin and the diffusion coefficient in the formulation of a drug are important factors that affect the percutaneous absorption kinetics of a drug. These parameters have been determined separately in various experiments. Partitioning from a formulation to skin has been estimated by measuring the tissue distribution of a drug (Cross et al., 2003; He et al., 2003; Thomas et al., 2004). The diffusion coefficient in a formulation has been determined from *in vitro* release studies (Higuchi, 1961; Higuchi, 1962; Chen-Chow and Frank, 1981; Upadrashta et al., 1993; Lu and Jun, 1998). The combined efficacy of these parameters on the skin permeation of a drug is complicated but analysis based on a diffusion model is thought to be useful in clarifying the efficacy of the parameters on skin permeation resulting from alteration of the formulation composition.

Several diffusion models have been developed to analyze skin permeation of a drug (Okamoto et al., 1989; Sugibayashi et al., 1996; Bando et al., 1996; Boderke et al., 2000; Kasting, 2001; Yamaguchi et al., 2006) in which "a well-stirred finite-dose condition" or "an infinite-dose condition" in a vehicle were assumed; however, diffusivity in a vehicle has not been

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considered. Guy and Hadgraft (1980) simulated the effect of diffusion in formulation on the skin permeation of a drug based on a mathematical model in their theoretical study. However, little is known about the relationship between diffusivity in a formulation and skin permeation in a practical study because, in almost all of the cases, skin permeation of the drug has been analyzed assuming a well-stirred condition in the formulation.

The aim of this study is to evaluate skin permeation of 22-oxacalcitriol (OCT) using two ointments of differing composition and discuss the variance of the skin permeation profiles of the ointments based on a diffusion model. OCT is a vitamin D_3 analogue (Abe et al., 1987) and the active ingredient of Oxarol®, an ointment used externally in the treatment of psoriasis (Barker et al., 1999). We previously reported on the *in vitro* rat skin permeation of OCT and the development of an analysis method based on a diffusion model including a metabolic process to estimate skin permeation parameters of OCT, which converts to several metabolites in skin (Yamaguchi et al., 2006). In the present study, an *in vitro* diffusion study and an *in vitro* rat skin permeation study of OCT were carried out using two ointments having differing solubilizer content, and the diffusion coefficients in the ointments and skin permeability were compared. Improving upon our previous diffusion model, we developed a two- or three-layer diffusion model including diffusivity in a vehicle to analyze the skin permeation of OCT applied as an ointment in which drugs would diffuse. Using the diffusion model, skin permeation parameters of OCT were estimated and two ointments were compared. Moreover, the effect of diffusivity in a formulation on the skin permeation of OCT was numerically evaluated.

2. Materials and methods

2.1. Materials

OCT (Fig. 1) was synthesized by Chugai Pharmaceutical Co. (Tokyo, Japan). Medium chain triglyceride (MCT) was purchased from the Nisshin OilliO Group Co. Ltd. (Tokyo, Japan). White petrolatum was purchased from Kozakai Pharmaceutical Co. Ltd. (Tokyo, Japan). Fetal bovine serum was purchased from MP Biomedicals (Irvine, CA, USA).

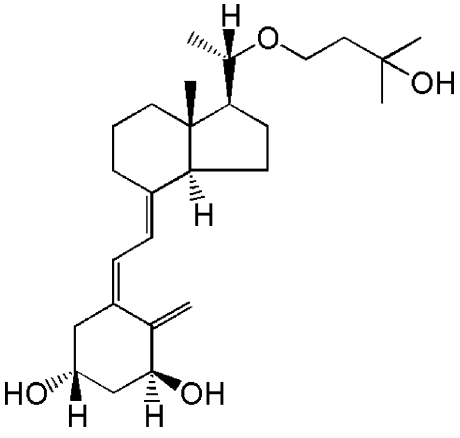

Fig. 1. Chemical structure of OCT.

2.2. Ointment preparation

Two types of OCT ointment were prepared by the same method as previously reported (Yamaguchi et al., 2006): one containing 3% (w/w) MCT (3MO) and the other containing 30% (w/w) MCT (30MO). The concentration of OCT in both ointments was set at 0.005% (w/w). 3MO was prepared using the following procedure. To 180 mg of MCT in a tube was added 300 μg of OCT as an ethanol solution, and the ethanol was evaporated under a nitrogen stream. To the MCT solution containing OCT, 5820 mg of white petrolatum was added. The tube was heated to 60 °C to melt the petrolatum and then the contents were mixed. When preparing 30MO, 1800 mg of MCT and 4200 mg of petrolatum were used and the same procedure was applied. Blank ointments, which had the same contents except without OCT, were also prepared. The ointments were stored at room temperature under shaded condition before use.

2.3. Diffusion study of OCT in ointments

Diffusion coefficients of OCT in the ointments were measured using a modified membraneless method with syringes (Lu and Jun, 1998). Fig. 2a shows the schematic illustration of this method. Blank and OCT ointments in separate syringes each with an inner area of 0.18 cm^2 were joined end to end with the ointments in contact at room temperature under shaded light. The syringes were separated after 5 days and the ointments were ejected from the syringes, cut into blocks, and sequentially labeled. Each block was weighed and dissolved in 3 ml of *n*-hexane. To the *n*-hexane solution, 3 ml of acetonitrile and 50 μl of 40 μg/ml *p*-hydroxybenzoic acid as the internal standard were added and mixed. The acetonitrile phase was collected and evaporated under a nitrogen stream. The extract was

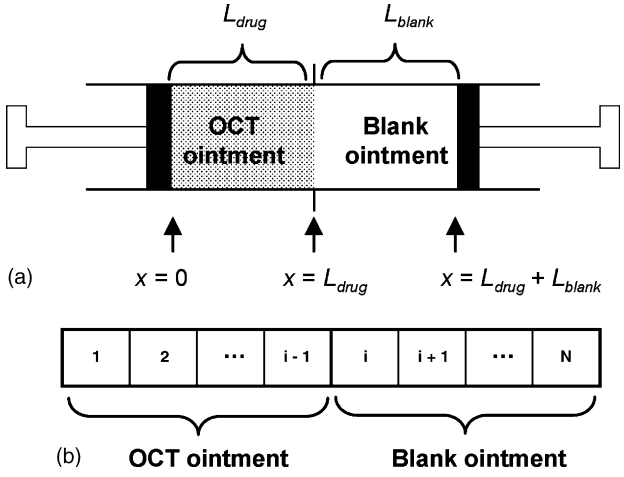

Fig. 2. Schematic representation of the modified membraneless method to determine diffusivity of a drug in ointment. Illustration of modified membraneless method (a). After incubation, the ointment from each syringe was ejected and cut into several blocks (b). The drug concentrations in the blocks were measured to obtain the concentration gradients.

dissolved in 250 μl of *n*-hexane/ethanol (85:15, v/v) to obtain samples for HPLC analysis and the amount of OCT in each sample was quantified. The HPLC apparatus consisted of a pump (LC-10AD$_{VP}$; Shimadzu Co., Kyoto, Japan), a UV detector (SPD-10A$_{VP}$; Shimadzu Co.), and an analytical column (TSK-gel OH-120, 4.6 mm × 250 mm; TOSOH Co., Tokyo, Japan). The mobile phase was *n*-hexane/ethanol (85:15, v/v) at a flow rate of 1 ml/min. The wavelength of the UV detector was set at 265 nm. The HPLC samples were injected at a volume of 50 μl.

2.4. In vitro rat skin permeation experiment

The care of the rats and the present protocols complied with the "General Consideration for Animal Experiments" and was approved by the Ethics Committee for Treatment of Laboratory Animals at Chugai Pharmaceuticals. Male rats (Sprague–Dawley strain, 7–8 weeks old; Japan SLC Inc., Hamamatsu, Japan) were used for the experiments. The rats were sacrificed under ether anesthesia, the dorsal region of the rat skin was carefully shaved and excised, and subcutaneous tissue was removed with scissors. To prepare the stripped skin, tape stripping was done 10 times before being excised. A polypropylene ring (inner depth: 1.6 cm, inner area: 2.0 cm^2) was glued to the epidermal side of the excised skin with cyanoacrylate adhesive.

Netwell^{TM} six-well plates (diameter: 24 mm, mesh size: 500 μm; Corning, MA, USA) were used for the skin permeation experiments (van de Sandt et al., 2000). Each well was filled with 2.0 ml of phosphate buffered saline (pH 7.4) containing 20% (v/v) fetal bovine serum as the receptor fluid. The excised rat skin was set dermis-side down on a Netwell^{TM} inserter in contact with the receptor fluid and placed in a CO_2 incubator at 37 °C. After 30 min of incubation, 15 mg (volume: 0.018 cm^3) of OCT ointment was applied to 2.0 cm^2 of skin surface. At 1, 4, 7, or 24 h after application, the ointment was wiped off with cotton, and the receptor fluid and the skin were collected. For the skin permeation experiments that continued for 24 h, receptor fluid was sampled at a volume of 1.0 ml at 2, 4, 6, 8, 20, and 24 h after application and 1.0 ml of fresh receptor fluid was added after each sampling.

2.5. Measurement of OCT in permeation experiment samples

OCT amounts in ointment, skin, and receptor fluid were determined by the same method as we previously reported (Yamaguchi et al., 2006). To the ointment sample, 15 ml of *n*-hexane was added, mixed, and extracted with 15 ml of acetonitrile. The acetonitrile layer was evaporated under a nitrogen stream. The extract was dissolved in 250 μl of *n*-hexane/*iso*-propanol/methanol (135:8:2, v/v/v) to obtain samples for HPLC analysis. The HPLC apparatus consisted of a pump (LC-10AD$_{VP}$), a UV detector (SPD-10A$_{VP}$), and an analytical column (YMC-Pack SIL A-004, 4.6 mm × 300 mm; YMC Co. Ltd., Kyoto, Japan). The mobile phase was *n*-hexane/*iso*-propanol/methanol (135:8:2, v/v/v) at a flow rate of 2.0 ml/min. The wavelength of the UV detector was set at 265 nm. The HPLC samples were injected at a volume of 50 μl.

To the skin sample, 70 ml of 70% (v/v) methanol was added and then homogenized. The supernatant of the homogenate was recovered, the extraction procedure was done once again, and the total volume of the skin extract was measured. As an internal standard, 20 μl of 500 ng/ml calcitriol was added to part of the skin extract and evaporated under a nitrogen stream; 0.5 ml of water and 2 ml of diethyl ether were added to the residue and the sample was mixed. The organic phase was evaporated under a nitrogen stream. The extract was dissolved in 50 μl of methanol/10 mM ammonium acetate (85:15, v/v) to obtain a LC–MS/MS sample in order to measure drug concentration. The LC–MS/MS analysis was carried out by coupling an HP1090 liquid chromatograph system (Hewlett Packard, Palo Alto, CA, USA) to an API300 mass spectrometer (Applied Biosystems/MDS SCIEX, Concord, Ont., Canada). A CAPCELL Pack C_{18} column (UG120, 2.0 mm × 150 mm; Shiseido Co. Ltd., Tokyo, Japan) was used as the analytical column. The mobile phase was methanol/10 mM ammonium acetate (85:15, v/v) at a flow rate of 0.2 ml/min. The HPLC eluent was introduced into the source using a TurboIonSpray® interface and the mass spectrometer was operated in the positive ion mode. Selected ions were *m/z* 297.3 (daughter ion of 436.3) for OCT and *m/z* 363.3 (daughter ion of 434.3) for calcitriol.

To the receptor fluid sample were added a three- to four-fold volume of diethyl ether and 20 μl of 500 ng/ml calcitriol as an internal standard. After mixing the sample, the ether phase was collected and evaporated, and the extract was dissolved in 50 μl of methanol/10 mM ammonium acetate (85:15, v/v) to obtain samples for LC–MS/MS analysis. The OCT concentration in the receptor fluid was measured using the same LC–MS/MS system as described above.

2.6. Analysis of in vitro diffusion study

In this study, the drug concentration in ointment is expressed as the following dimensionless equation:

$$\frac{\partial C'(y,t)}{\partial t} = D'_{V}\frac{\partial^2 C'(y,t)}{\partial y^2}$$

where y is equal to x/L_{total} and $C'(y,t)$ is equal to $C(x,t)L_{total}$. $C(x,t)$ is the drug concentration (μg/cm^3) at position x at time t after jointing syringes and L_{total} is the sum of length (cm) of the drug ointment (L_{drug}) and the blank ointment (L_{blank}). The initial conditions of the OCT ointment and the blank ointment are $C(x,0) = C_0$ $(0 \le x \le L_{drug})$ and $C(x,0) = 0$ $(L_{drug} < x \le L_{drug} + L_{blank})$, respectively, where C_0 is the initial drug concentration in ointment. D'_V is equal to D_V/L^2_{total} and D_V is the diffusion coefficient (cm^2/h) in the ointment. The partial differential equation is transformed to a time-dependent ordinary differential equation by the method of lines, solved by the implicit EULER rule.

As shown in Fig. 2b, the OCT ointment and the blank ointment lie to the left and right side, respectively, and the ointment is divided into several blocks. The amount of OCT ($Q_i(t)$) in the *i*th ointment block from the left side at time t is expressed as the

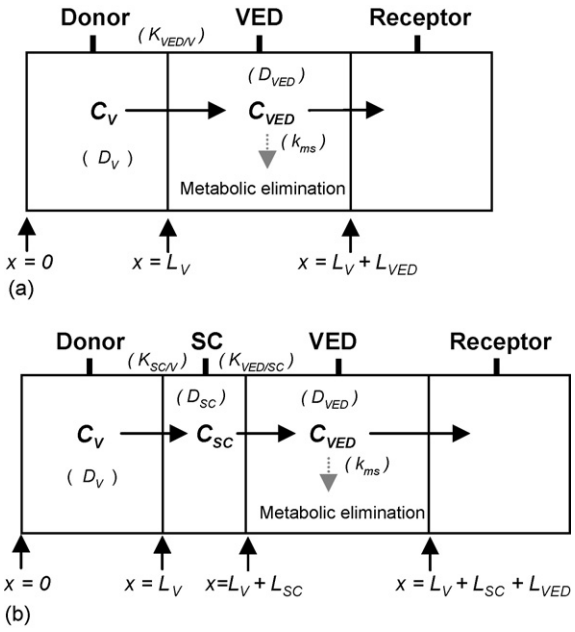

Fig. 3. Schematic representation of a diffusion model for skin permeation of a drug through stripped skin (two-layer diffusion model) (a) and full-thickness skin (three-layer diffusion model) (b).

following equation:

$$Q_i(t) = \text{area} \int_a^b C'(y,t)\,\mathrm{d}y, \qquad a = \sum_{k=1}^{i-1} \left(\frac{L_\mathrm{k}}{L_\mathrm{total}} \right),$$

$$b = \sum_{k=1}^{i} \left(\frac{L_\mathrm{k}}{L_\mathrm{total}} \right) \quad (a = 0 \text{ when } i = 1)$$

where L_i is the length of the ith ointment block from the left side (Fig. 2b) and area is the inner area of the syringe.

Each $Q_i(t)$ was fitted to the corresponding observed data by a nonlinear least squares regression program MULTI (Yamaoka et al., 1981), and D'_V (=$D_\mathrm{V}/L^2_\mathrm{total}$) was calculated and transformed to D_V.

2.7. Analysis of skin permeation of OCT based on a diffusion model including diffusivity in vehicle

2.7.1. Model for stripped skin (two-layer diffusion model)

Fig. 3 shows a schematic representation of the diffusion model expressing skin permeation of a drug. Assuming that: (1) the behavior of a drug in vehicle and viable epidermis and dermis (VED) obey Fick's law of diffusion, (2) the drug is metabolized in VED at a rate of first order kinetics, and (3) sink condition in the receptor fluid, the following dimensionless partial differential equations are derived for the stripped skin model.

$$\frac{\partial C'_\mathrm{V}(y,t)}{\partial t} = D'_\mathrm{V} \frac{\partial^2 C'_\mathrm{V}(y,t)}{\partial y^2}, \quad (0 < y < 1);$$

$$\frac{\partial C'_\mathrm{VED}(y,t)}{\partial t} = D'_\mathrm{VED} \frac{\partial^2 C'_\mathrm{VED}(y,t)}{\partial y^2} - k_\mathrm{ms} C'_\mathrm{VED}(y,t),$$

$$(1 < y < 2)$$

The initial and boundary conditions are expressed as the following equations:

At $t = 0$

$$C'_\mathrm{V}(y,t) = C_{\mathrm{V}0} L_\mathrm{V}, \quad (0 \le y \le 1);$$

$$C'_\mathrm{VED}(y,t) = 0, \quad (1 < y \le 2)$$

At $t > 0$

$$D'_\mathrm{V} \frac{\partial C'_\mathrm{V}(y,t)}{\partial y} = D'_\mathrm{VED} \frac{\partial C'_\mathrm{VED}(y,t)}{\partial y},$$

$$K'_\mathrm{VED/V} C'_\mathrm{V}(y,t) = C'_\mathrm{VED}(y,t), \quad (y = 1);$$

$$C'_\mathrm{VED}(y,t) = 0, \quad (y = 2)$$

where y is equal to x/L_V $(0 \le x \le L_\mathrm{V})$ and x/L_VED $(L_\mathrm{V} < x \le L_\mathrm{V} + L_\mathrm{VED})$. $C'_\mathrm{V}(y,t)$ and $C'_\mathrm{VED}(y,t)$ are equal to $C_\mathrm{V}(x,t)L_\mathrm{V}$ and $C_\mathrm{VED}(x,t)L_\mathrm{VED}$, respectively. C_V (x,t) and C_VED (x,t) are drug concentrations in the vehicle and VED, respectively, at position x at time t after application. $C_{\mathrm{V}0}$ is the initial drug concentration in the vehicle. L_V and L_VED are the thickness of the vehicle and VED, respectively. D'_VED is equal to $D_\mathrm{VED}/L^2_\mathrm{VED}$, where D_VED is the diffusion constant in VED. $K'_\mathrm{VED/V}$ is equal to $K_\mathrm{VED/V}L_\mathrm{VED}/L_\mathrm{V}$, where $K_\mathrm{VED/V}$ is the partition coefficient at the interface of the VED/vehicle. k_ms is the metabolic elimination constant in VED. The partial differential equations described above are transformed to time-dependent ordinary differential equations by the method of lines, solved by the implicit EULER rule.

The amount of drug in the ointment ($Q_\mathrm{V}(t)$), in skin ($Q_\mathrm{S}(t)$), and in the receptor fluid ($Q_\mathrm{R}(t)$) at time t is calculated by the following equations:

$$Q_\mathrm{V}(t) = \text{area} \int_0^1 C'_\mathrm{V}(y,t)\,\mathrm{d}y \tag{1}$$

$$Q_\mathrm{S}(t) = \text{area} \int_1^2 C'_\mathrm{VED}(y,t)\,\mathrm{d}y \tag{2}$$

$$Q_\mathrm{R}(t) = -D'_\mathrm{VED}\,\text{area} \int_0^t \frac{\partial C'_\mathrm{VED}(y,t)}{\partial y}\mathrm{d}t|_{y=2} \tag{3}$$

where *area* is the effective diffusion area.

2.7.2. Model for full-thickness skin (three-layer diffusion model)

In the full-thickness skin model, stratum corneum (SC) is added to the stripped skin model as a diffusion layer having no metabolic activity. The following dimensionless partial differential equations are derived.

$$\frac{\partial C'_\mathrm{V}(y,t)}{\partial t} = D'_\mathrm{V} \frac{\partial^2 C'_\mathrm{V}(y,t)}{\partial y^2} \quad (0 < y < 1);$$

$$\frac{\partial C'_\mathrm{SC}(y,t)}{\partial t} = D'_\mathrm{SC} \frac{\partial^2 C'_\mathrm{SC}(y,t)}{\partial y^2}, \quad (1 < y < 2);$$

$$\frac{\partial C'_{\mathrm{VED}}(y,t)}{\partial t} = D'_{\mathrm{VED}}\frac{\partial^2 C'_{\mathrm{VED}}(y,t)}{\partial y^2} - k_{\mathrm{ms}}C'_{\mathrm{VED}}(y,t),$$

$(2 < y < 3)$

The initial and boundary conditions are expressed as the following equations:

At $t=0$

$C'_{\mathrm{V}}(y,t) = C_{\mathrm{V0}}L_{\mathrm{V}}, \quad (0 \le y \le 1);$

$C'_{\mathrm{SC}}(y,t) = 0, \quad (1 < y \le 2);$

$C'_{\mathrm{VED}}(y,t) = 0, \quad (2 < y \le 3)$

At $t>0$

$$D'_{\mathrm{V}}\frac{\partial C'_{\mathrm{V}}(y,t)}{\partial y} = D'_{\mathrm{SC}}\frac{\partial C'_{\mathrm{SC}}(y,t)}{\partial y},\ K'_{\mathrm{SC/V}}C'_{\mathrm{V}}(y,t) = C'_{\mathrm{SC}}(y,t),$$

$$(y=1); \qquad D'_{\mathrm{SC}}\frac{\partial C'_{\mathrm{SC}}(y,t)}{\partial y} = D'_{\mathrm{VED}}\frac{\partial C'_{\mathrm{VED}}(y,t)}{\partial y},$$

$K'_{\mathrm{VED/SC}}C'_{\mathrm{SC}}(y,t) = C'_{\mathrm{VED}}(y,t), \quad (y=2);$

$C'_{\mathrm{VED}}(y,t) = 0, \quad (y=3)$

where y is equal to x/L_{V} $(0 \le x \le L_{\mathrm{V}})$, x/L_{SC} $(L_{\mathrm{V}} < x \le L_{\mathrm{V}} + L_{\mathrm{SC}})$ and x/L_{VED} $(L_{\mathrm{V}} + L_{\mathrm{SC}} < x \le L_{\mathrm{V}} + L_{\mathrm{SC}} + L_{\mathrm{VED}})$. $C'_{\mathrm{SC}}(y,t)$ is equal to $C_{\mathrm{SC}}(x,t)L_{\mathrm{SC}}$. $C_{\mathrm{SC}}(x,t)$ is the drug concentration at position x at time t, and L_{SC} is the length of SC. D'_{SC} is equal to $D_{\mathrm{SC}}/L^2_{\mathrm{SC}}$, where D_{SC} is the diffusion constant in SC. $K'_{\mathrm{SC/V}}$ and $K'_{\mathrm{VED/SC}}$ are equal to $K_{\mathrm{SC/V}}L_{\mathrm{SC}}/L_{\mathrm{V}}$ and $K_{\mathrm{VED/SC}}L_{\mathrm{VED}}/L_{\mathrm{SC}}$, respectively. $K_{\mathrm{SC/V}}$ and $K_{\mathrm{VED/SC}}$ are the partition coefficients at the interface of SC/vehicle and VED/SC, respectively. The partial differential equations described above are transformed to time-dependent ordinary differential equations by the method of lines, solved by the implicit EULER rule.

The amount of drug in the ointment ($Q_{\mathrm{V}}(t)$), in skin ($Q_{\mathrm{S}}(t)$), and in receptor fluid ($Q_{\mathrm{R}}(t)$) at time t are calculated using the following equations:

$$Q_{\mathrm{V}}(t) = \text{area}\int_0^1 C'_{\mathrm{V}}(y,t)\,\mathrm{d}y \tag{4}$$

$$Q_{\mathrm{S}}(t) = \text{area}\int_1^2 C'_{\mathrm{SC}}(y,t)\,\mathrm{d}y + \text{area}\int_2^3 C'_{\mathrm{VED}}(y,t)\,\mathrm{d}y \tag{5}$$

$$Q_{\mathrm{R}}(t) = -D'_{\mathrm{VED}}\,\text{area}\int_0^t \frac{\partial C'_{\mathrm{VED}}(y,t)}{\partial y}\,\mathrm{d}t|_{y=3} \tag{6}$$

2.7.3. Data analysis

Drug amount in donor (Q_V) was obtained by HPLC analysis. Drug amount in skin (Q_S) was calculated by the following equation: Q_S = (drug concentration in skin extract) × (Skin extract volume). Cumulative drug amount permeated into the receptor fluid (Q_R) was calculated by the following equation:

$$Q_{\mathrm{R}} = C_i V + \sum_{k=1}^{i-1} C_k v \quad (Q_{\mathrm{R}} = C_i V \quad \text{when } i = 1)$$

where i is the sampling number, and C_i is the drug concentration in receptor fluid at the ith sampling time. v and V are the sampling volume and total volume of receptor fluid, respectively.

Mean values of Q_{V}, Q_S and Q_{R} of three experiments at each sampling time were calculated. First, the permeation profiles of OCT through stripped skin were fitted to Eqs. (1)–(3) using MULTI (Yamaoka et al., 1981). As a result, the parameters (i.e., $K'_{\mathrm{VED/V}}$, D'_{VED} and k_{ms}) were calculated. Next, the permeation profiles of OCT through full-thickness skin were fitted to Eqs. (4)–(6) using the D'_{VED} value obtained from stripped skin analysis. As a result, the parameters (i.e., $K'_{\mathrm{SC/V}}$, $K'_{\mathrm{VED/SC}}$ $(= K'_{\mathrm{VED/V}}/K'_{\mathrm{SC/V}})$ and D'_{SC}) were calculated, and k_{ms} was recalculated. Because the surface of stripped skin was considered to be damaged and the metabolic activity might have changed, the recalculated k_{ms} value was adopted as the actual metabolic rate constant. In the case of analyzing the skin permeation of OCT through full-thickness skin after application of 30MO, Q_{V} and Q_{R} were fitted to Eqs. (4) and (6), respectively, using parameters ($K'_{\mathrm{VED/SC}}$, D'_{VED}, and k_{ms}) obtained from the skin permeation analysis of OCT with 3MO. From the results, the parameters (D'_{SC} and $K'_{\mathrm{SC/V}}$) with 30MO were calculated.

The parameters D'_{SC}, D'_{VED}, $K'_{\mathrm{VED/V}}$, $K'_{\mathrm{SC/V}}$, and $K'_{\mathrm{VED/SC}}$ were transformed to D_{SC}, D_{VED}, $K_{\mathrm{VED/V}}$, $K_{\mathrm{SC/V}}$, and $K_{\mathrm{VED/SC}}$, respectively, assuming L_{SC}: 0.001 cm, L_{VED}: 0.1 cm, and L_{V} (ointment volume/application area): 0.009 cm. The permeability coefficients through SC (P_{SC}) and VED (P_{strip}) were calculated using the following equations:

$$P_{\mathrm{SC}} = \frac{K_{\mathrm{SC/V}}D_{\mathrm{SC}}}{L_{\mathrm{SC}}}, \qquad P_{\mathrm{strip}} = \frac{K_{\mathrm{VED/V}}D_{\mathrm{VED}}}{L_{\mathrm{VED}}}.$$

2.8. Simulation study to evaluate the effect of diffusivity in ointment on skin permeation

Using the skin permeation parameters of OCT obtained from the skin permeation experiment with 3MO and assuming several diffusion coefficients in ointment, the effect of diffusivity in ointment on the skin permeation of OCT was simulated. The cumulative amount and residual drug amount in ointment were calculated based on a two- or three-layer diffusion model considering diffusivity in ointment using diffusion coefficients in the range of 1×10^{-5} to 1×10^{6} cm^2/h. In all simulations, the experimental conditions of the *in vitro* skin permeation experiment of OCT were used (application area: 2 cm^2, ointment volume: 0.018 cm^3).

3. Results and discussion

3.1. Diffusion study of OCT in ointments

Fig. 4 shows the diffusion coefficients of OCT in the two ointments. The diffusion coefficients of OCT in 3MO and 30MO were 0.89 ($\pm$0.07) $\times 10^{-4}$ cm^2/h and 1.87 ($\pm$0.11) $\times$ 10^{-4} cm^2/h, respectively. An increase of MCT content in the ointment caused an increase in the diffusion coefficient of OCT, which was significantly different ($p < 0.01$, unpaired Student's

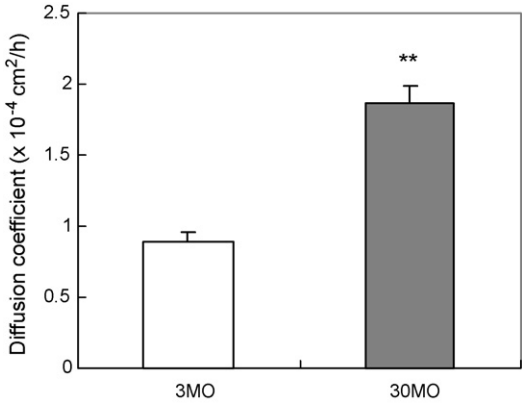

Fig. 4. Diffusion coefficients of OCT in two ointments. Diffusion coefficients were determined by a modified membraneless method under room temperature ($n = 3$). Error bars show S.D. 3MO: OCT ointment containing 3% (w/w) MCT, 30MO: OCT ointment containing 30% (w/w) MCT. $^{**}p < 0.01$ (unpaired Student's *t*-test).

t-test) between the two ointments. High diffusivity in 30MO might be due to the high fluidity of the ointment.

3.2. In vitro rat skin permeation study

Fig. 5 shows the *in vitro* rat skin permeation profile of OCT. In the case of 3MO, 7% of the applied OCT dose permeated through full-thickness skin by 24 h after application and 22% remained in the ointment. Using stripped skin, more rapid OCT absorption was observed: 31% of the applied dose permeated through skin by 24 h after application, and only 7% remained in the ointment at 7 h after application. In the case of 30MO, 2% of the applied dose permeated through full-thickness skin by 24 h after application and 65% remained in the ointment. An increase of MCT content in the ointment caused a decrease of OCT absorption. Although it was expected that high diffusivity in the ointment would cause an increase in skin permeation, the cumulative amount of OCT permeated was higher with 3MO than with 30MO, contrary to our expectations.

The total unchanged OCT in the experimental system (ointment, skin, and cumulative permeated) 24 h after application of

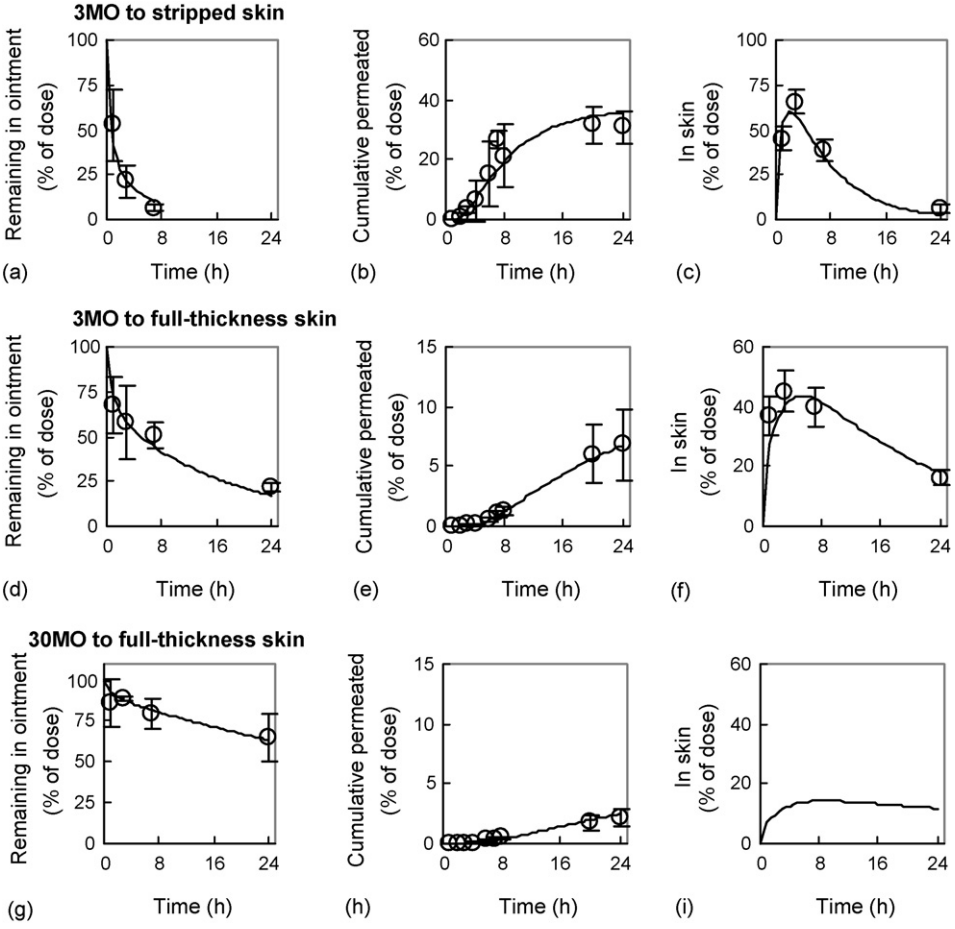

Fig. 5. *In vitro* rat skin permeation profiles of OCT after application of 3MO and 30MO. Application of 3MO to stripped skin: amount of OCT remaining in ointment (a), cumulative amount permeated (b), and amount in skin (c). Application of 3MO to full-thickness skin: amount of OCT remaining in ointment (d), cumulative amount permeated (e), and amount in skin (f). Application of 30MO to full-thickness skin: amount of OCT remaining in ointment (g), cumulative amount permeated (h), and amount in skin (i). Open circles: the mean values of observed data; error bars: S.D. of observed data; solid lines: simulation curves based on a diffusion model.

3MO to full-thickness skin and stripped skin were 45% and 37% of the applied dose, respectively. We previously reported that almost all of the radioactivity was recovered from the *in vitro* rat skin permeation experimental system when [^{3}H]OCT was applied and that OCT was converted to several metabolites in rat skin (Yamaguchi et al., 2006). These results suggest that the decrease in total unchanged OCT from the experimental system was mainly caused by continual metabolic elimination in skin, not by low recoveries.

3.3. Analysis of skin permeation of OCT based on a diffusion model

We previously reported that OCT was highly metabolized in rat skin. The skin permeation of OCT was successfully analyzed based on a diffusion model considering metabolic processes, assuming that the time dependent decrease of unchanged OCT was mainly caused by metabolic elimination in skin and a well-stirred condition in the ointment (Yamaguchi et al., 2006). In this study, we improved our previous diffusion model and developed a two- or three-layer diffusion model considering diffusivity in vehicle in order to analyze the effect of formulation on skin permeation parameters such as diffusivity in a formulation, diffusivity in skin, and partitioning.

Simulation curves obtained by the diffusion model had good correlation with corresponding observed data (Fig. 5), suggesting the diffusion models were suitable to express the skin permeation profile of OCT. The observed skin permeation profiles of OCT were correlated with the corresponding simulated data which were calculated by a diffusion model assuming the first-order kinetics metabolism in skin. This suggests that the metabolic activity in skin was approximated to the first-order kinetics and that the OCT metabolism was not saturated. Although the OCT amount in skin was not determined in the experiment using 30MO, the OCT amount in skin with 30MO must be lower than that with 3MO as shown in Fig. 5 (i) as follows: (1) the decrease of OCT from ointment with 30MO was slower than that with 3MO, and (2) the cumulative OCT permeated through the skin with 30MO was lower than that with 3MO.

Table 1 summarizes the permeation parameters of OCT calculated by the curve-fitting procedure. The metabolic activity in stripped skin ($k_{ms,st}$: 1.09×10^{-1} h^{-1}) was 0.3-fold of that in full-thickness skin (k_{ms}: 3.41×10^{-1} h^{-1}), suggesting that the metabolic activity in stripped skin decreased due to the damage caused by tape-stripping. The diffusion constant, D_{VED}, was 4.04×10^{-4} cm^2/h. The diffusion constants, D_{SC}, with 3MO and 30MO were 7.45×10^{-8} cm^2/h and 6.92×10^{-8} cm^2/h, respectively. The D_{SC} values differed little between 3MO and 30MO. Considering the standard deviations of the estimated parameters (Table 1), no significant difference was found in D_{SC} between formulations. The partition coefficient $K_{VED/SC}$ was 5.23×10^{-2}, indicating SC has higher affinity to OCT than VED. The $K_{SC/V}$ value of OCT with 3MO was 1.16×10^{1} and five-fold higher than 30MO, suggesting that the difference in partitioning to SC caused the difference in the skin permeation rate. Ceschel et al. (2002) reported that partition coefficients of ketoprofen to skin were inversely proportional to their solubility in vehicles. It was considered that the low solubilizer concentration caused low solubility of OCT in the ointment and high partitioning to SC because MCT is a solubilizer of OCT. It is not clear whether MCT changed the OCT solubility in SC. However, the rate of change of OCT solubility caused by MCT must be smaller in SC than the ointment because an increase of MCT in the ointment caused a decrease of OCT partitioning to SC. If the rate of change of OCT solubility in SC was the same as that in the ointment, the partitioning to SC would not be affected by the MCT content in the ointment.

In this study, it was assumed that no change was observed in the permeation parameters (k_{ms}, $K_{VED/SC}$, and D_{VED}) which were related to the drug transport in the layer below SC, and these parameters were obtained from the analysis of the experiments using 3MO. Although $K_{SC/V}$ and D_{SC} values were obtained from the analysis of full-thickness skin experiments with 3MO and 30MO, only the $K_{SC/V}$ value was changed between 3MO and 30MO, i.e., the difference in the full-thickness skin permeation profiles between formulations could be explained by the difference in $K_{SC/V}$. This result suggests that the assumption was correct and that MCT affected only the interface of skin surface/vehicle ($K_{SC/V}$) but not the transport in both the inner SC (D_{SC}) and the lower layer ($K_{VED/SC}$, D_{VED}, and k_{ms}).

The permeability coefficients P_{SC} were 8.62×10^{-4} cm/h with 3MO and 1.77×10^{-4} cm/h with 30MO. P_{strip} was 2.44×10^{-3} cm/h with 3MO. The permeation resistances in SC ($1/P_{SC}$) and VED ($1/P_{strip}$) with 3MO were 1161 and 409 h/cm, respectively, $1/P_{strip}$ was 0.35-fold of $1/P_{SC}$. This suggests that SC is the main permeation barrier for OCT and VED also behaves as a permeation barrier (Yamaguchi et al., 2006). In this study, the ointment (0.018 cm^3) was applied to a 2-cm^2 surface area of skin. The ointment was applied to the skin surface horizontally, the ointment thickness (L_V) was calculated to be 0.009 cm, and the permeability coefficient in ointment (D_V/L_V) was calculated to be 9.89×10^{-3} cm/h with 3MO. The permeability coefficient of OCT through full-thickness skin, P_{full}

Table 1
In vitro rat skin permeation parameters of OCT estimated by a diffusion model considering diffusivity in a vehicle

Ointment	Parameters								
	$K_{VED/V}$ ($\times 10^{-1}$)	$K_{SC/V}$	$K_{VED/SC}$ ($\times 10^{-2}$)	D_{SC} ($\times 10^{-8}$ cm^2/h)	D_{VED} ($\times 10^{-4}$ cm^2/h)	k_{ms} ($\times 10^{-1}$ h^{-1})	$k_{ms,st}$ ($\times 10^{-1}$ h^{-1})	P_{SC} ($\times 10^{-4}$ cm/h)	P_{strip} ($\times 10^{-3}$ cm/h)
3MO	6.04 (0.93)	11.6 (1.2)	5.23 (0.97)	7.45 (0.93)	4.04 (0.35)	3.41 (0.46)	1.09 (0.17)	8.62	2.44
30MO	–	2.37 (0.57)	5.23 (0.97)	6.92 (2.00)	4.04 (0.35)	3.41 (0.46)	–	1.77	–

Values in parenthesis are S.D. for estimated parameters; –: not determined.

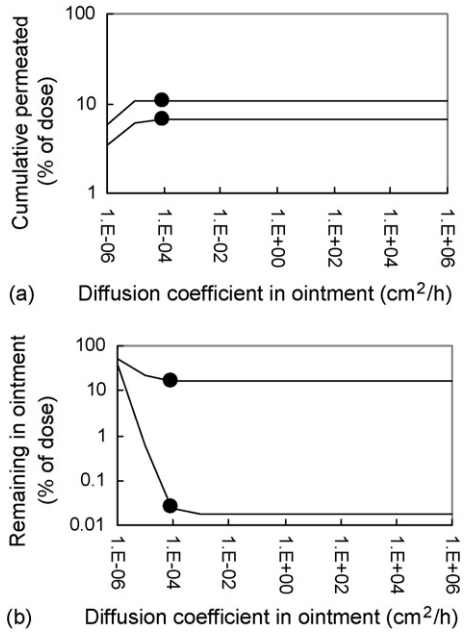

Fig. 6. Simulated permeation profiles of OCT at 24 h after application assuming various diffusion coefficients in ointment: simulation of cumulative amount permeated (a), simulation of amount remaining in ointment (b). Solid lines: stripped skin, dotted lines: full-thickness skin, closed circles: simulated value using intact diffusion coefficient of OCT in 3MO.

(Ghanem et al., 1992), was calculated by the following equation:

$$\frac{1}{P_{\text{full}}} = \frac{1}{P_{\text{SC}}} + \frac{1}{P_{\text{strip}}}$$

In the case of 3MO, P_{full} and P_{strip} were 6.37×10^{-4} and 2.44×10^{-3} cm/h, respectively. $D_{\text{V}}/L_{\text{V}}$ was 16-fold higher than P_{full} and four-fold higher than P_{strip}, indicating that the permeability of OCT through ointment was higher than through full-thickness or stripped skin.

3.4. Effect of diffusion coefficient in ointment on OCT permeation—simulation study

Using skin permeation parameters of OCT obtained from skin permeation experiments with 3MO and assuming several diffusion coefficients in an ointment, the cumulative amount permeated and the drug amount remaining in an ointment at 24 h after application were simulated to evaluate the effect of diffusivity in ointment on skin permeation of OCT. Assuming full-thickness skin permeation, both the cumulative amount and remaining amount were constant when the diffusion coefficient in the ointment was more than 1×10^{-5} cm^2/h, as shown in Fig. 6. Assuming stripped skin permeation, the cumulative amount and remaining amount were constant when the diffusion coefficients were more than 1×10^{-5} and 0.89×10^{-4} cm^2/h, respectively. According to the simulation experiment, the diffusion coefficient of OCT in 3MO (0.89×10^{-4} cm^2/h) was high enough to have no affect on the cumulative amount of OCT permeated or the remaining amount in the ointments. Although a simulation experiment assuming skin permeation of OCT with 30MO was not carried out, the following two results indicate that the diffusion coefficient of OCT in 30MO was also high enough to be ineffective: (1) the diffusion coefficient in 30MO was higher than 3MO and (2) P_{SC} with 30MO was lower than that with 3MO.

4. Conclusions

In this study, *in vitro* rat skin permeation of OCT was evaluated using two ointments having differing MCT contents, and skin permeation profiles were analyzed successfully based on a two- or three-layer diffusion model considering diffusivity in an ointment. The parameters (D_{SC}, D_{V} and $K_{\text{SC/V}}$) that are possibly altered by the formulation composition were estimated and compared between the two ointments. The following results were obtained: (1) D_{SC} values with 3MO and 30MO were the same, (2) $K_{\text{SC/V}}$ with 3MO was five-fold greater than with 30MO, and (3) D_{V} values had a two-fold difference but both were high enough not to affect skin permeation of OCT. These findings suggest that the differences between the skin permeation profiles of the two OCT ointments were mainly caused by the difference in $K_{\text{SC/V}}$ but not D_V and that an increase of MCT content in an OCT ointment causes a decrease in the skin permeation of OCT due to the decrease of $K_{\text{SC/V}}$ in the range of 3–30% (w/w).

Analysis combined with simulation of the present diffusion model enabled us to divide the cause of skin permeation alteration from formulation into diffusivity in a formulation, partitioning, and diffusivity in skin and to clarify which parameter had more effect on skin permeation. In the previous analysis methods for skin permeation of a drug, a well-stirred condition in the donor was assumed and the methods were mainly used to analyze skin permeation using a liquid type vehicle as a donor. On the other hand, in the present analysis, both diffusivity and an infinite dose condition in the donor were assumed. Thus, the present method is applicable to the analysis of skin permeation profiles using practical formulations, especially sustained release preparations. This would be useful for setting a target value of diffusivity in a formulation in the development of controlled-release preparations.

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