ELSEVIER

International Journal of Pharmaceutics 135 (1996) 127–136

international journal of pharmaceutics

Analysis of in vitro rat skin permeation and metabolism of SM-10902, prodrug of synthetic prostacyclin analogue

Kimihiko Sato*, Takeshi Mine

Research Center, Sumitomo Pharmaceuticals Co., Ltd., 1-98, Kasugade Naka-3, Konohana-ku, Osaka 554, Japan

Received 30 March 1995; revised 28 October 1995; accepted 9 January 1996

Abstract

The permeation and metabolism of SM-10902, a prodrug of a synthetic prostacyclin analogue (SM-10906), in the rat skin were studied using a flow through type diffusion cell and were compared with those of SM-10906. Absorbed SM-10902 was entirely metabolized to the bioactive form, SM-10906, in the rat skin. The appearance of SM-10906 to the receptor was faster when applied as SM-10902 than when applied as SM-10906 in both types of intact and stripped skin. From the analysis of these permeation profiles by the two-layer skin model with a metabolic pathway, the diffusion constants of SM-10902 in the stratum corneum and the lower layer were 70 and 6 times of those of SM-10906, respectively. On the other hand, the partition coefficient of SM-10902 from the ointment to the stratum corneum and the lower layer were equal to and twice as high as those of SM-10906, respectively. Furthermore, to clarify the suitable characteristic for skin permeation, the relationship between the metabolic rate in the lower layer and the permeation profile of the ester prodrug was estimated by computer simulation. The appearance of metabolite to the receptor increases with the increase of metabolic rate and reaches a maximum point and thereafter decreases to a plateau level. According to this simulation, it is shown that SM-10902 has a favorable characteristic from the view point of metabolic rate.

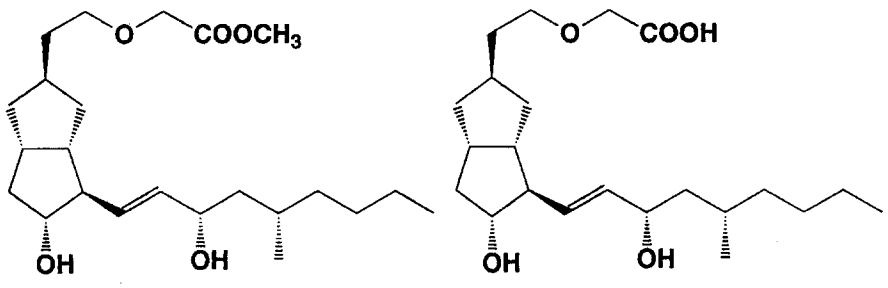
Keywords: Skin permeability; Skin metabolism; Prodrug; In vitro; Prostaglandin

1. Introduction

The vasodilating activity of prostaglandins is expected to cure the peripheral circulation insufficiency and the transdermal application is useful to prevent the side effect of systemically-applied prostaglandins such as diarrhea, headache (Lewis et al., 1981), and steal phenomenon (Coffman, 1979), and to maintain the drug concentration at the therapeutic site. However, the skin is known to act as a barrier for environmental invasion, preventing sufficient permeation of the drug. Therefore, many approaches have been made to improve the skin permeability of the drug, i.e. permeation enhancers, iontophoresis and chemical modification.

* Corresponding author.

PII S0378-5173(95)04446-8



SM-10902 **SM-10906**

Fig. 1. Chemical structures of SM-10902 and SM-10906.

SM-10902, (+)-methyl[2-[(2R,3aS,4R,5R,6aS)-octahydro-5-hydroxy-4-[(E)-(3S,5S)-3-hydroxy-5-methyl-1-nonenyl]-2-pentalenyl]ethoxy]acetate, and its demethylated form SM-10906 are chemically-stable prostaglandin derivatives (Fig. 1). We previously reported that SM-10902 applied to guinea pig auricles increased the skin temperature but neither SM-10906 nor PGI_2 did (Yamamoto et al., 1994). However, SM-10906 has a much higher effect on the in vitro thrombin-induced increase in the intracellular Ca^{2+} concentration than SM-10902 (Oka et al., 1994). These findings indicate that SM-10902 is a prodrug of SM-10906 and permeates into the skin more effectively than SM-10906 and shows its pharmacologic activity after deesterification by tissue esterase.

In this study, we examined the permeation characteristics of SM-10902 and SM-10906 using the in vitro diffusion cell method and show the potential of SM-10902 as a topical application.

2. Materials and methods

2.1. Materials

SM-10906, SM-10902 and propylester of SM-10906 were synthesized in our laboratory. White Vaseline and sililation reagents (N-ethyl-N-nitrosourea, N,O-Bis(trimethylsilyl)trifluoro-acetoamide (BSTFA), pyridine and acetonitrile) were purchased from Nacalai Tesque, INC. (Japan). Hydroxypropyl methyl cellulose was purchased from Aldrich Chemical Co., Inc. (USA). Penicillin G potassium salt was purchased from Banyu Pharmaceutical Co., Ltd. (Japan). Streptomycin sulfate was purchased from Sigma Chemical Co. (USA). All other chemicals were of analytical grade.

2.2. Octanol/water partition coefficient (log P)*

About 8 mg of SM-10906 or SM-10902 was dissolved in 0.2 ml of n-octanol and mixed with 3 ml of 1/15M phosphate buffer (pH 7.4). The mixture was shaken for 1 h at room temperature for equilibration. Then the two phases were separated by centrifugation at 10000 rpm for 5 min. Using the HPLC apparatus, the drug concentration of the n-octanol phase was determined after dilution with acetonitrile and that of the aqueous phase was determined without dilution. The HPLC apparatus was composed of LC-6A pump (Shimadzu Co., Japan), a SPD-6A spectrophotometer (Shimadzu Co.) monitoring the absorbance at 210nm and a reversed phase Sumipax ODS A-212 column (6 mm i.d. × 15 cm, Sumika Chemical Analysis Service, Ltd., Japan). The mobile phase consisted of 30mM phosphoric acid adjusted to pH3.0 by triethylamine and acetonitrile (35:65, v/v) was maintained at a flow rate of 1 ml/min.

2.3. Percutaneous permeation experiment

Skin permeation experiments were performed according to the method of Okamoto et al.

(1986). The dorsal hair of a male SD rat (7 weeks old, CREA Inc. Japan) was removed carefully using hair clippers. Full thickness intact skin, consisting of stratum corneum, viable epidermis and dermis, was obtained after sacrifice. Stripped skin was prepared by 15-times tape stripping and photo-microscopically confirmed that the skin was composed of viable epidermis and dermis (data not shown). Excised skin was mounted on the flow through type diffusion cell (diffusion area: 1.13 cm^2) and the receptor was perfused with isotonic phosphate buffer containing 0.5%(w/v) methyl cellulose, 50 mg/l streptomycin sulfate and 30 mg/l penicillin G potassium salt at a flow rate of 1 ml/h. After 2 h of preperfusion, 25 mg of Vaseline ointment containing 1% of each drug was applied to the donor side. The ointment was formulated by dissolving the drug to melted Vaseline (about 50°C). The receptor fluid was collected every 1 h for 24 h. Throughout the experiment, the room temperature was kept at 25°C. The concentration of SM-10902 and SM-10906 in the receptor fluids were measured by GC/MS as follows. The collected receptor fluid was mixed with 20 μl of formic acid and 56 ng of propyl-SM-10906 as the internal standard. The mixture was extracted twice with 4 ml of n-hexane containing 3% isoamyl alcohol. The organic layer was evaporated to dryness. The residue was esterified with 1 ml of diazoethane in ether at an ambient temperature for 1 h and the reaction mixture was evaporated to dryness. To the residue, 50 μl each of BSTFA, pyridine and acetonitrile were added and the mixture was trimethylsilylated at 60°C for 30 min. Then the reaction mixture was evaporated to dryness. The residue was redissolved in 50 μl of acetonitrile and analyzed by HP5988A-5890 GC/MS system (Hewlett-Packard Co., USA) equipped with DB-1 column(2.5 mm i.d. × 15 m, thickness 0.25 μm, J & W Co., Ltd., USA). Using this GC/MS system, SM-10906 and SM-10902 were simultaneously measured and the detection limit was about 2ng/ml for each drug.

3. Theoretical

To clarify the permeation profile of both the prodrug and the bioactive form, we took into account the deesterification of the prodrug in the viable epidermis and dermis to develop the two-layer skin model (Okamoto et al., 1989).

3.1. Diffusion model for SM-10902 in intact skin

Fig. 2A shows the diffusion model for SM-10902 in the intact skin. SM-10902 partitions to the stratum corneum (thickness:L_s) from the ointment with a partition coefficient k_{2s} and diffused in the stratum corneum with a diffusion constant D_{2s}. At the end of stratum corneum, SM-10902 partitions to the lower layer (viable epidermis and dermis) with a partition coefficient k_{2v}. SM-10902 diffuses in the lower layer (thickness:L_v) with a diffusion constant D_{2v} and is de-esterified to SM-10906. To simplify the skin model, the metabolic reaction in the lower layer was assumed to be first order with the rate constant (ke). The generated SM-10906 diffuses in the lower layer with a diffusion constant D_{6v}. At the end of the lower layer, SM-10902 and SM-10906 is cleared by the perfusion medium with a constant clearance CL_2 and CL_6, respectively. Here, it is assumed that the amount of SM-10906 partitioned from the lower layer to the stratum corneum is negligible.

Therefore, the diffusion of SM-10902 and SM-10906 in the intact skin can be described by Fick's second law of diffusion as:

$$\frac{\partial C_{2s}}{\partial t} = D_{2s} \cdot \frac{\partial^2 C_{2s}}{\partial x^2}$$

$$\frac{\partial C_{2v}}{\partial t} = D_{2v} \cdot \frac{\partial^2 C_{2v}}{\partial x^2} - k_e \cdot C_{2v}$$

$$\frac{\partial C_{6v}}{\partial t} = D_{6v} \cdot \frac{\partial^2 C_{6v}}{\partial x^2} + k_e \cdot C_{2v}$$

where C_{2s} and C_{2v} are the drug concentration of SM-10902 in the stratum corneum and the lower layer, respectively; C_{6v} is the drug concentration of SM-10906 in the lower layer. The initial conditions are as follows:

$$C_{2s} = C_{2v} = C_{6v} = 0$$

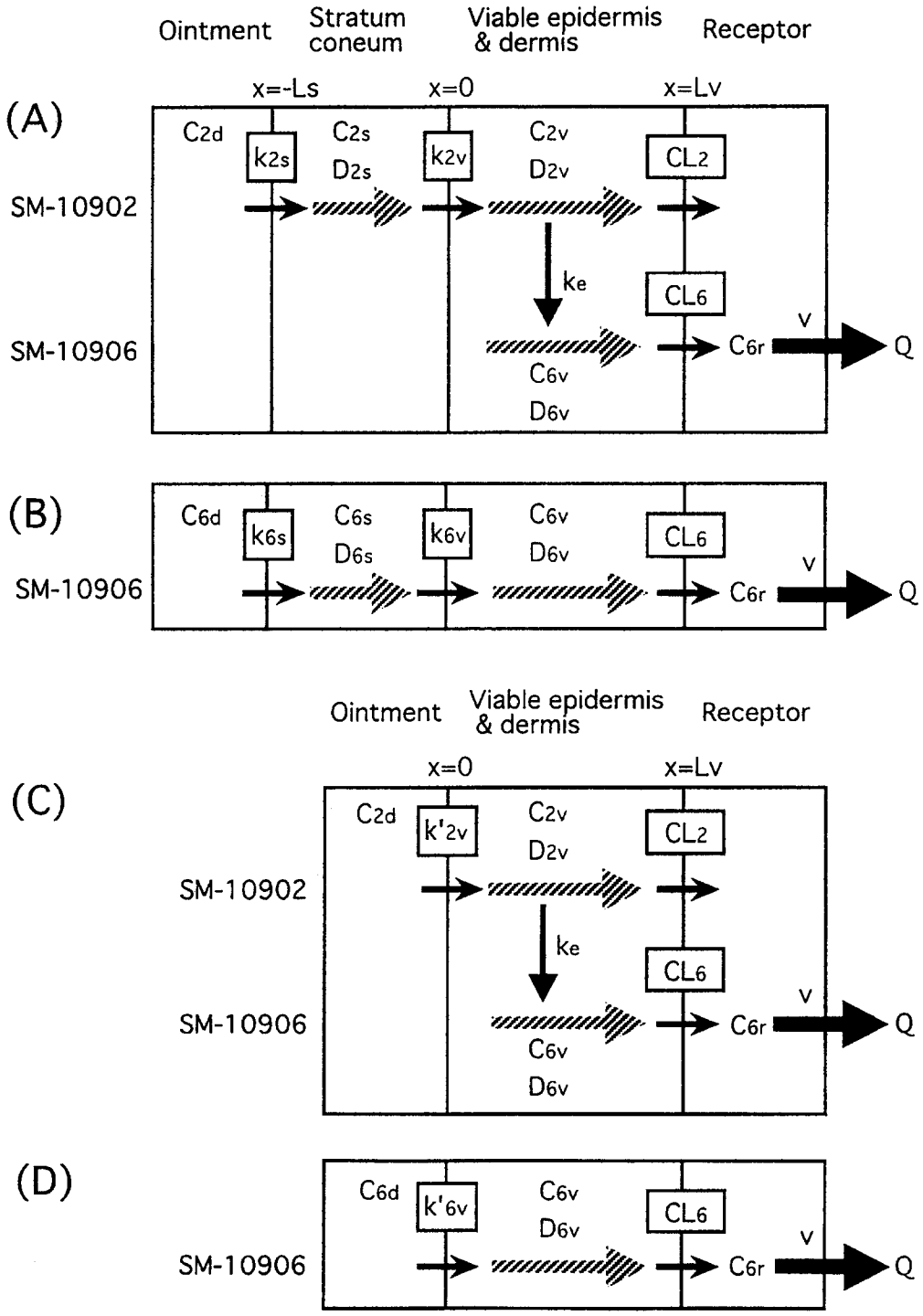


Fig. 2. Schematic diagram of the two layer skin model for intact skin (panel A: SM-10902, panel B: SM-10906) and stripped skin (panel C: SM-10902, panel D: SM-10906). Keys: C, concentration; D, diffusion constant; k or k', partition coefficient; CL, clearance; v, perfusion speed; x, distance; Q, cumulative amount of SM-10906 permeated. Subscripts 2 and 6 mean the drug species, SM-10902 and SM-10906, respectively. Subscripts d, s, v and r mean the compartment, ointment (dose), stratum corneum, viable epidermis and dermis and receptor, respectively.

Here, the clearance CL_2 is considered to be nearly zero from the experimental results and as assuming that the donor is under an infinite condition (C_{2d} is constant) and the receptor is under a well-stirred sink condition, the boundary conditions are given as follows:

$$k_{2s} \cdot C_{2d} = C_{2s}\,(x = -L_s)$$

$$k_{2v} \cdot C_{2s} = C_{2v}\,(x = 0)$$

$$D_{2s} \cdot \frac{\partial C_{2s}}{\partial x} = D_{2v} \cdot \frac{\partial C_{2v}}{\partial x}\,(x = 0)$$

$$CL_2 \cdot C_{2v} = -D_{2v} \cdot \frac{\partial C_{2v}}{\partial x}\,(x = L_v,\ CL_2 = 0)$$

$$\frac{\partial C_{6v}}{\partial x} = 0\,(x = 0)$$

$$CL_6 \cdot C_{6v} = -D_{6v} \cdot \frac{\partial C_{6v}}{\partial x}\,(x = L_v)$$

The mass balance at the receptor and the cumulative amount (Q) of SM-10906 appeared in the receptor is expressed as follows:

$$Vr \cdot \frac{dC_{6r}}{dt} = Sa \cdot CL_6 \cdot \{C_{6v}\}_{x = L_v} - v \cdot C_{6r}$$

$$Q = \int_0^t v \cdot C_{6r}\,dt$$

where C_{6r} is the concentration of SM-10906 at the receptor; Vr is the receptor volume; Sa is the surface area for diffusion; and v is the perfusion speed.

From these equations, the Laplace transform for the cumulative amount of SM-10906 after topical application of SM-10902 is expressed as follows:

$$\bar{Q} = \frac{Sa \cdot CL_6 \cdot v \cdot A}{s \cdot Vr + v} \quad (1)$$

where s is the Laplace operator with respect to time; and

$$A = \frac{\beta \cdot \sinh(\beta \cdot L_v) - \gamma \cdot \sinh(\gamma \cdot L_v)}{E \cdot \gamma^2 \cdot \cosh(\beta \cdot L_v)} \cdot G \cdot C$$

$$E = CL_6 \cdot \cosh(\gamma \cdot L_v) + D_{6v} \cdot \gamma \cdot \sinh(\gamma \cdot L_v)$$

$$G = \frac{k_e}{D_{6v} \cdot \beta^2 - s}$$

$$C = \frac{k_{2s} \cdot k_{2v} \cdot \beta \cdot C_{2d} \cdot \cosh(\beta \cdot L_v)}{s \cdot \beta \cdot \cosh(-\alpha \cdot L_s) \cdot \cosh(\beta \cdot L_v) - (s + k_e) \cdot \alpha \cdot k_{2v} \cdot \sinh(-\alpha \cdot L_s) \cdot \sinh(\beta \cdot L_v)}$$

$$\alpha = \sqrt{\frac{s}{D_{2s}}} \quad \beta = \sqrt{\frac{s + k_e}{D_{2v}}} \quad \gamma = \sqrt{\frac{s}{D_{6v}}}$$

3.2. Diffusion model for SM-10902 in stripped skin

Fig. 2C shows the diffusion model for SM-10902 at the stripped skin. SM-10902 partitions to the lower layer from the ointment with a partition coefficient k'_{2v}. The fate of SM-10902 in the stripped skin is the same as that in the lower layer after the application of SM-10902 to the intact skin. Therefore, the diffusion of SM-10902 and SM-10906 in the stripped skin are described by Fick's second law of diffusion, and the Laplace transform for the cumulative amount of SM-10906 is also expressed as equation (1) with the same abbreviations except for C. In this case, abbreviation C is given as follows:

$$C = \frac{k'_{2v} \cdot C_{2d}}{s}$$

3.3. Diffusion model for SM-10906 in intact skin

Fig. 2B shows the diffusion model for SM-10906 in the intact skin. The disposition of SM-10906 in the intact skin is the same as in the application of SM-10902 except for no metabolic pathway and is expressed using the partition coefficients from the ointment to stratum corneum (k_{6s}), from stratum corneum to the lower layer (k_{6v}) and the diffusion constant in the stratum corneum (D_{6s}).

Therefore, the diffusion of SM-10906 in the intact skin is described by Fick's second law of diffusion and the Laplace transform for the cumulative amount of SM-10906 appeared in the receptor after topical application of SM-10906 is expressed as follows:

$$\bar{Q} = \frac{Sa \cdot CL_6 \cdot v \cdot B}{s \cdot Vr + v} \quad (2)$$

where

$$B = \frac{-k_{6s} \cdot k_{6v} \cdot C_{6d}}{s \cdot \{\delta \cdot k_{6v} \cdot \sinh(-\delta \cdot L_s) \cdot E - \gamma \cdot \cosh(-\delta \cdot L_s) \cdot F\}}$$

$$E = CL_6 \cdot \cosh(\gamma \cdot L_v) + D_{6v} \cdot \gamma \cdot \sinh(\gamma \cdot L_v)$$

$$F = D_{6v} \cdot \gamma \cdot \cosh(\gamma \cdot L_v) + CL_6 \cdot \sinh(\gamma \cdot L_v)$$

$$\gamma = \sqrt{\frac{s}{D_{6v}}} \quad \delta = \sqrt{\frac{s}{D_{6s}}}$$

3.4. Diffusion model for SM-10906 in stripped skin

Fig. 2D shows the diffusion model for SM-10906 at stripped skin. SM-10906 partitions to the lower layer from the ointment with a partition coefficient k'_{6v}. The fate of SM-10906 in the stripped skin is the same as that in the lower layer after the application of SM-10906 to the intact skin. So, the diffusion of SM-10906 in the stripped skin is described by Fick's second law of diffusion and the Laplace transform for the cumulative amount of SM-10906 is also expressed as equation (2) with the same abbreviations except for B. In this case, abbreviation B is given as follows:

$$B = \frac{k'_{6v} \cdot C_{6d}}{F \cdot \gamma \cdot s}$$

4. Data analysis

To obtain the disposition parameters, permeation profiles were fitted to equation (1) and (2) using MULTI-FILT (Yano et al., 1989) by three steps. At the first step, the permeation data after the application of SM-10906 to the stripped skin was analyzed to obtain D_{6v}, CL_6 and k'_{6v}. At the second step, the parameters obtained in the first step were fixed and the permeation data after the application of SM-10906 to the intact skin was analyzed. At the third step, the parameters obtained in the first step were fixed and the permeation data after the application of SM-10902 to the stripped and the intact skin were simultaneously analyzed, because stepwise analysis of these two data resulted in unsuccessful fitting of the latter data. In fitting calculation, to simplify, the skin model the effective diffusion length in the stratum corneum and the lower layer were assumed to be equal to the physical length and were fixed to 0.002 and 0.1cm, respectively (Robert et al., 1983; Sato et al., 1991).

5. Result and discussion

5.1. Physicochemical properties

SM-10906, the bioactive form, with a pKa value of 3.9 (data not shown) is ionized under physiological pH and the apparent partition coefficient (log P*) between n-octanol and phosphate buffer (pH 7.4) is 0.8. On the other hand, the partition coefficient of SM-10902, prodrug of SM-10906, which has no ionizable functional group is 4.5. Therefore, the lipophilicity of the prodrug is increased about a 1000-fold as compared with the bioactive form.

The skin permeability is generally dependent on the lipophilicity of the substances (Flynn et al., 1981). Therefore, the large difference in log P* will reflect the difference of the permeation profile of both drugs.

5.2. Percutaneous permeation characteristics

After the application of SM-10902 or SM-10906 to intact or stripped skin, only SM-10906 was detected in the perfusion medium. Especially in the case of the application of SM-10902, SM-10902 was not detected (< 2 ng/ml) in the receptor fluid, even when the concentration of SM-10906 in the receptor fluid exceeds 2000 ng/ml. Because SM-10902 was stable in the ointment and in the perfusion medium for the experimental period, it was suggested that absorbed SM-10902 was entirely de-esterified to SM-10906 in the skin,

Table 1
Diffusion parameters of SM-10902 and SM-10906 in the rat skin

Compound	Stratum cornenm		Viable epidermis and dermis			
	Diffusion constant (cm^2/h)	Partition coefficient	Diffusion constant (cm^2/h)	Partition coefficient	Clearance ($ml/h/cm^2$)	Metabolic rate (h^{-1})
SM-t0906	4.10×10^{-6}	2.56×10^{-4}	4.67×10^{-4}	$1.1\,2 \times 10^{-2}$	1.17	—
SM-10902	2.79×10^{-4}	2.76×10^{-4}	2.69×10^{-3}	2.65×10^{-2}	—	0.17

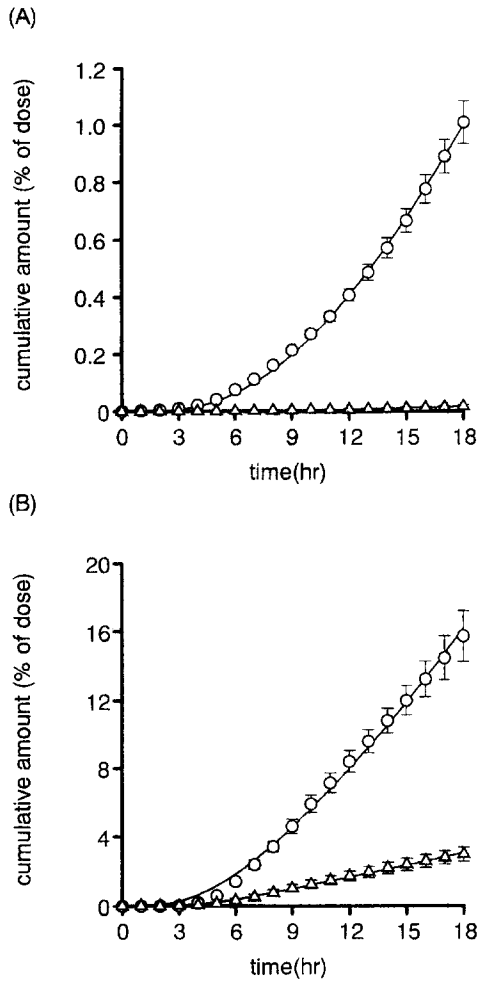


Fig. 3. Simulated and measured cumulative amount of SM-10906 appeared in the receptor after the application of SM-10902 (○) and SM-10906 (△) to the rat intact (panel A) and stripped (panel B) skin. The solid lines represent the cumulative amount of SM-10906 simulated by the two-layer skin model with metabolic pathway. Each value is expressed as the mean ± s.e. of four or five experiments.

probably in the lower layer (Yu et al., 1980; Tojo et al., 1994). Fig. 3 shows the cumulative amount of SM-10906 which appeared in the receptor after the application of SM-10902 or SM-10906 to the intact and the stripped skin. The amount of appeared SM-10906 was more abundant after the application of SM-10902 than that of SM-10906 in both types of skin, and markedly increased by removal of stratum corneum.

The cumulative amount of both drugs was analyzed by the skin model and the calculated best fit curves are shown in Fig. 3 as the solid lines. Good correlations were observed between experimental data and calculated cumulative amount. Table 1 summarizes the diffusion parameters of SM-10902 and SM-10906 in the skin. Because SM-10902 was not detected in the receptor fluid, we assumed $CL_2 = 0$ to establish the skin model. To certify the validity of this hypothesis, we estimated the maximum CL_2 value (1.07×10^{-5} ml/h/cm^2) when the concentration of SM-10902 in the receptor fluid dose not exceed the detection limit. The difference of the calculated cumulative amount of SM-10906 at 18 h after the application of SM-10902 to the stripped skin between the two cases of $CL_2 = 0$ and 1.07×10^{-5} was less than 0.008% of the dose (0.05% of the cumulative amount). So the hypothesis ($CL_2 = 0$) was considered to be reasonable.

The diffusion constants of SM-10902 in the stratum corneum and the lower layer were 70 and 6 times of those of SM-10906, respectively. On the other hand, the partition coefficient of SM-10902 from the ointment to the stratum corneum and the lower layer were equal to and twice as high as those of SM-10906, respectively. This means that the increase in lipophilicity by methyl esterification leads to the more permeable properties such as the increases in both the diffusion constants and the partition coefficients. The large difference in the diffusion constants between SM-10902 and SM-10906 was perplexing from the view point of the similar molecular weight of these drugs. However, there are many reports to show that several permeation pathways exist in the stratum corneum and the dominant pathway is determined by the physicochemical properties of the substrates (Tojo, 1987; Prakash et al., 1988; Yamashita et al., 1993). In the case of SM-10902 and SM-10906, they had greatly different physicochemical properties from one another, such as the lipophilicity and whether there was an ionizable functional group or not under the physiological condition. This evidence suggested the existence of a different pathway in the stratum corneum for each drug.

In spite of the large difference in log P*, there was a little difference in the partition coefficient from the ointment to the stratum corneum of both drugs. This was due to the difference in the partitioning manner between two systems. That was, log P* was the oil to water partition coefficient, while the partition coefficient at the skin was thought to be like a oil to oil partition coefficient. So, the difference in the partition coefficient at the skin was not necessary to reflect the difference in log P*.

The metabolic rate of SM-10902 to SM-10906 in the lower layer, which is not able to be measured directly, was calculated to about 0.17 h^{-1} by using the skin model. Furthermore, to clarify the relationship between the metabolic rate in the lower layer and the permeation profile of ester prodrug, the permeation profiles of SM-10906 after the application of SM-10902 with various metabolic rate were simulated in the intact skin (Fig. 4). The cumulative amount increases with the increase of metabolic rate (the rate limiting process of this phase is the generation of SM-10906) and reaches the vertex point (around 1 h^{-1}) and thereafter decreases to plateau (the rate limiting process of this phase is the diffusion of SM-10906 in the lower layer). The cumulative amount of SM-10906 at 18 h after the application of SM-10902 to intact skin (1% of applied dose) was 70% of the maximum value and 80% of the plateau value.

From this study, it is shown that the appearance of the active metabolite to the receptor after the application of the prodrug is determined not only by the partition coefficients and the diffusion constants but also by the metabolic rate. It is also shown that SM-10902 is more advantageous in diffusion and partitioning at the skin than SM-10906 and has a preferable metabolic rate in the skin.

Acknowledgements

We wish to thank Dr. Kiyoshi Yamaoka for providing us the computer program MULTI(-FILT). We also would like to thank Dr. Toshiyuki Hirohashi and Dr. Terushige Kato for

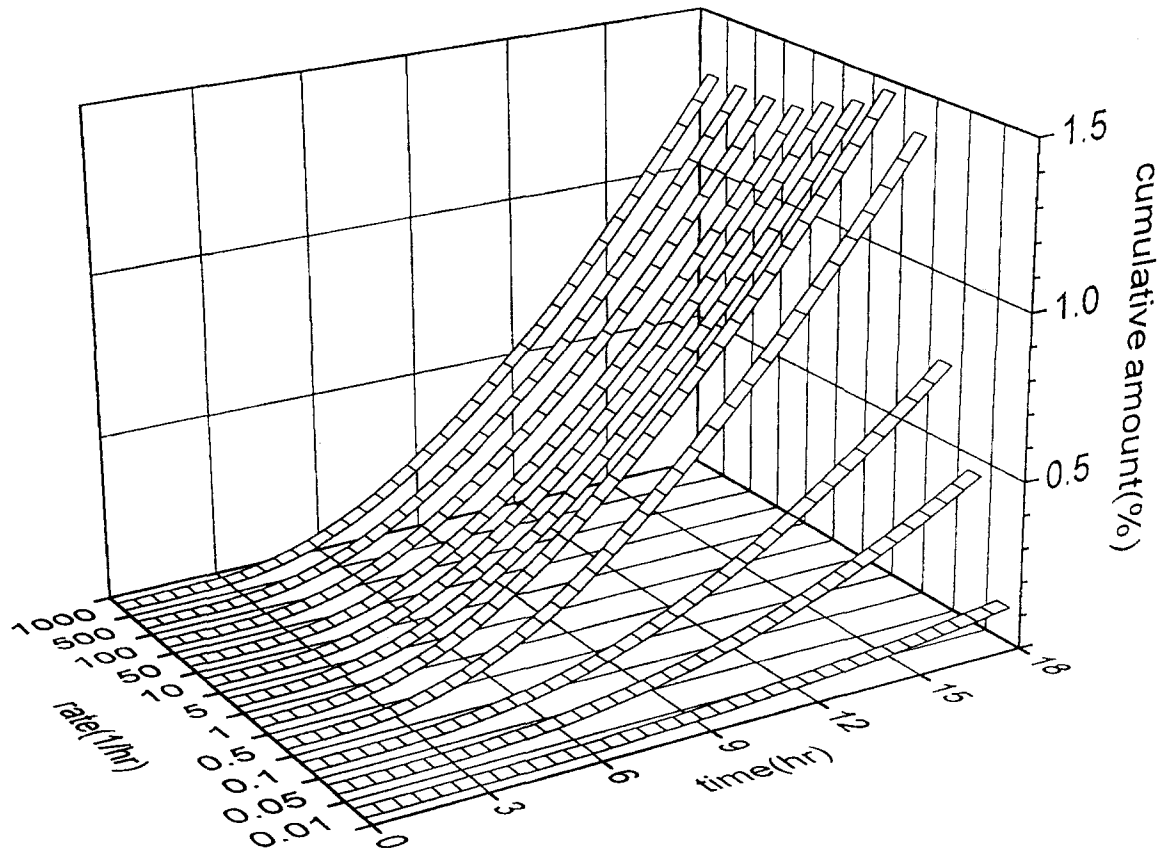


Fig. 4. The effect of metabolic rate on the simulated cumulative amount of SM-10906 after the application of SM-10902 to the intact skin.

helpful support during this work and Mr. Yoshihiro Yasui for technical assistance.

References

Flynn, G.L., Durrheim, H. and Higuchi, W.I., Permeation of hairless mouse skin II: Membrane sectioning techniques and influence on alkanol permeabilities. *J. Pharm. Sci.*, 70 (1981) 52–56.

Lewis, A.B., Freed, M.D., Heymann, M.A., Roehl, S.L. and Kensey, R.C., Side effects of therapy with prostaglandin E_1 in infants with critical congenital heart disease. *Circulation*, 64 (1981) 893–898.

Coffman, M.D., Vasodilator drugs in peripheral vascular disease. *N. Engl. J. Med.*, 300 (1979) 713–717.

Oka, M., Negishi, M., Yamamoto, T., Satoh, K., Hirohashi, T. and Ichikawa, A., Prostacyclin (PGI) receptor binding and cyclic AMP synthesis activities of PGI_1 analogues, SM-10906 and its methyl ester, SM-10902, in mastocytoma P-815 cells. *Biol. Pharm. Bull.*, 17 (1994) 74–77.

Okamoto, H., Komatsu, H., Hashida, M. and Sezaki, H., Effect of β-cyclodextrin and di-*O*-methyl-β-cyclodextrin on the percutaneous absorption of butylparaben, indomethacin and sulfanilic acid. *Int. J. Pharm.*, 30 (1986) 35–45.

Okamoto, H., Yamashita, F., Saito, K. and Hashida, M., Analysis of drug penetration through the skin by the two-layer skin model. *Pharm. Res.*, 6 (1989) 931–937.

Prakash, V.R., Man-Cheong, F. and Bradley, D.A., The role of protein and lipid domains in the uptake of solutes by human stratum corneum. *Pharm. Res.*, 5 (1988) 140–150.

Robert, L.B., Raymond, F.S. and Elaine, R.C., Difference in permeability of rat skin related to sex and body site. *J. Soc. Cosmet. Chem.*, 34 (1983) 127–135.

Sato, K., Sugibayashi, K. and Morimoto, Y., Species differences in percutaneous absorption of nicorandil. *J. Pharm. Sci.*, 80 (1991) 104–107.

Tojo, K., Random brick model or drug transport across stratum corneum. *J. Pharm. Sci.*, 76 (1987) 889–891.

Tojo, K., Yamada, K. and Hikima, T., Diffusion and metabolism of predonisolone farnesylate in viable skin of the hairless mouse. *Pharm. Res.*, 11 (1994) 393–397.

Yamamoto, T., Satoh, K., Nishimura, T., Horikawa, N., Mine, T., Hirohashi, T. and Hara, Y., Pharmacological properties of the new stable prostacyclin analogue 3-oxo-methano-prostaglandin I_1. *Arzneim. Forsch.*, 44 (1994) 483–490.

Yamashita, F., Yoshioka, T., Koyama, Y., Okamoto, H., Sezaki, H. and Hashida, M., Analysis of skin penetration enhancement based on a two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum: Dose-dependent effect of 1-geranylazacycloheptan-2-one on drugs with different lipophilicities. *Biol. Pharm. Bull.*, 16 (1993) 690–697.

Yano, Y., Yamaoka, K. and Tanaka, H., A nonlinear least squares program, MULTI(FILT), based on fast Laplace transform for microcomputers. *Chem. Pharm. Bull.*, 37 (1989) 1035–1038.

Yu, C.D., Fox, J.L., Higuchi, W.I. and Ho, N.F.H., Physical model evaluation of topical prodrug delivery-simultaneous transport and bioconversion of vidarabine-5'-valerate IV: Distribution of esterase and deaminase enzymes in hairless mouse skin. *J. Pharm. Sci.*, 69 (1980) 772–775.