Penetration of Ufenamate into Intact, Stripped, or Delipidized Skin Using Different Vehicles

Hayato Iino,¹,² Makiko Fujii,*,¹,³ Manami Fujino,¹ Naoya Koizumi,¹ and Yoshiteru Watanabe¹

¹Department of Pharmaceutics and Biopharmaceutics, Showa Pharmaceutical University; 3–3165, Higashi-Tamagawagakuen, Machida, Tokyo 194–8543, Japan; and ²Research and Development Department, Yuskin Pharmaceutical Co., Ltd.; 1–1–11, Kaizuka, Kawasaki-ku, Kawasaki, 210–0014, Japan.

Received March 24, 2015; accepted July 28, 2015

The purpose of this study was to clarify the effect of skin condition on skin penetration of the very high lipophilic drug, ufenamate (UF). UF was applied to stripped or delipidized skin using liquid paraffin (LP) or purified water containing polysorbate 80 at a dose of 2µg/cm². We found that UF penetration into intact and stripped skin using a water vehicle was respectively 5 and 10 times higher than that using LP. UF is freely soluble in oil and insoluble in water; thus, activity in water is higher than that in LP. Therefore, it is useful to use a water-based vehicle for both intact sites and those with defective stratum corneum (SC). Conversely, we found that delipidization of SC decreased the penetration of UF significantly with both LP and water, and the amount measured in the epidermis was 1µg/cm² with both vehicles. This indicates that UF is not suitable for so-called “dry skin.” This study revealed clinically relevant differences in the penetration of UF into intact, stripped, or delipidized skin conditions.

Key words skin penetration; ufenamate; stripped skin; delipidized skin; oil vehicle; water vehicle

Skin penetration of drugs is widely studied as a way to deliver drugs to the systemic circulation. Transdermal therapeutic systems should be applied to intact skin. Conversely, dermal application of drugs for treating topical skin diseases is common. In these cases, the skin at the application site may not be intact due to the underlying disorder. The state of the skin, especially the stratum corneum (SC), which is the main barrier layer of the skin, is important for understanding drug penetration. However, there has been little discussion regarding skin penetration of drugs for topical use under different skin conditions.¹,²

Gujjar et al. reported the skin penetration of water-soluble drugs in different skin conditions;³ however, lipophilic drugs used in dermatology area have not been studied. In this study, skin penetration of ufenamate (UF) with different vehicles into skin with various conditions was studied. UF is an anthranilic acid-based anti-inflammatory drug developed for skin diseases, such as acute and chronic eczema, contact dermatitis, diaper dermatitis, miliaria and atopic dermatitis.⁴⁻⁸ These skin diseases are caused by peeling of SC or delipidized skin surface; however, the penetration of UF into the damaged skin has not been reported. We evaluated two types of damaged skin models, stripped skin (no SC barrier function) and delipidized skin (decreased SC intercellular lipids). It is well known that drug and vehicle characteristics affect skin penetration. UF is highly lipophilic with an octanol/water partition coefficient (log P) of 6.7. UF is freely soluble in oil and is poorly soluble in water. UF is used in the form of ointments or creams; thus, we used two types of vehicles to simplify the test material, liquid paraffin (LP) and water with surfactant.

MATERIALS AND METHODS

Materials Japanese Pharmaceutical Codex grade UF (molecular weight: 337.34) was obtained from Shiono Finesse (Osaka, Japan). LP and polysorbate 80 (TO) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Nikko Chemicals (TO-10MV, Tokyo, Japan), respectively. The other reagents used were of analytical or HPLC grade.

Skin Penetration Study Yucatan micropig (YMP) skin (Charles River Laboratories, Kanagawa, Japan) was used as intact skin with the adhering fat layer removed.⁹⁻¹⁰ Delipidized skin was prepared by applying 2mL of a 1 : 1 mixed solvent of acetone and ether to intact skin attached to a modified Franz-type diffusion cell apparatus for 40min.¹¹ YMP skin has about 20 layers of SC. Skin was stripped 50 times using adhesive tape (Scotch 313, 3M, Tokyo, Japan) to obtain complete SC removal (stripped skin).

UF dissolved in LP or emulsified in water with 3% TO at a concentration of 5% was used as the test material. A piece of skin was placed on a modified Franz-type diffusion cell apparatus (effective area, 1.1cm²) For receptor phase, 17mL of pH 7.1 phosphate-buffered saline (PBS) added 5% TO to improve the solubility of UF and keep sink condition, and was kept at 37°C. Test material (2mL) was poured into the donor phase, and the donor phase was closed to prevent evaporation of the sample solution (infinite condition). In the case of practical dose condition, 2µL of test material was applied to the skin, and the skin was placed on the diffusion cell. At predetermined times, 0.2mL of the receptor was withdrawn and the same volume of fresh PBS was added to maintain a constant volume.

After the skin penetration study, the skin surface was wiped with a laboratory wipe (KimWipes, Nippon Paper Cre-cia, Tokyo, Japan). The wipe was soaked in methanol in the cases of practical dose condition. Although SC is consisted to be composed of about 20 layers, outer 10 layers are easily

¹Present address: School of Pharmacy, Nihon University; 7–7–1 Narashinodai, Funabashi, Chiba 274–8555, Japan.

* To whom correspondence should be addressed. e-mail: fujii.makiko@nihon-u.ac.jp

© 2015 The Pharmaceutical Society of Japan
collected by stripping with adhesive tape. Furthermore, UF amount in less than 10 layers of SC was lower than the limit of quantification obtained using HPLC. Thus, skin was then stripped 10 times with adhesive tape to collect SC. Remaining skin was then separated into epidermis and dermis using the heat separation method. UF in stripped SC, epidermis, or dermis was extracted with methanol and UF concentration was determined by HPLC. UF collected from wipes and from the first and second strips of tape were considered to be on the surface. A data recovery percentage of UF over 75% of the applied amount was adopted in the case of practical dose conditions.

**HPLC Conditions** HPLC was performed using a LC-10A VP system (Shimadzu, Kyoto, Japan) equipped with Wako-sil-II5C18HG (150×4.6 mm; Wako Pure Chemical Industries, Ltd.) under the following conditions: mobile phase, methanol–purified water (90:10); column temperature, 25°C; flow rate, 1.0 mL/min; detection wavelength, 285 nm. Components of the adhesive tape were confirmed in advance so that they do not effect the determination of UF.

**Statistical Analyses** We used ANOVA followed by Fisher’s protected least significant difference test. A $p<0.05$ was considered statically significant.

**RESULTS AND DISCUSSION**

**Penetration of UF into Intact Skin** Until now, there have been few reports on the skin penetration of UF. Prior to examining penetration of UF into damaged skin, it is necessary to understand the penetration of UF into intact skin. The skin penetration of UF with LP and water into intact skin was studied under various application conditions. Figures 1, 2 show the amount of UF in the skin under infinite (a) and practical dose conditions (b) after application as LP or water system, respectively. UF was not observed at the receptor phase regardless of the vehicle or application condition. It was reported that blood concentrations of UF, detected as $^{14}$C, were low after skin application in rats in vivo because lipophilic drugs hardly

![Fig. 1. Effect of Application Conditions on Penetration of Ufenamate into Intact Skin with Liquid Paraffin](image)

(a) Infinite; (b) Practical dose condition. Application period: □, 4h; ■, 48h. The data show mean±S.D. of at least 4 experiments.

![Fig. 2. Effect of Application Conditions on Penetration of Ufenamate into Intact Skin with Water](image)

(a) Infinite; (b) Practical dose condition. Application period: □, 4h; ■, 48h. The data show mean±S.D. of at least 4 experiments. Significantly different ($p<0.05$) from infinite 4h.
permeate the hydrophilic layer, epidermis.\(^{13}\)

After application as an LP solution, amounts of UF in SC and epidermis showed no difference between application periods of 4 and 48h and their concentrations in the dermis increased (Fig. 1). This indicates that the penetration of UF in SC and epidermis reached steady state at 4h. UF amounts in the epidermis and dermis tended to be lower under the practical dose condition than under infinite conditions, but it was not significant. The effects of the application period and amount were the same with water as with LP (Fig. 2). In practical dose conditions, water should be evaporated from test material; however, UF was saturated in test material so that there was no difference because of the application amount. Thus, our further studies used the practical dose condition.

**Penetration of UF into Damaged Skin** Figure 3 shows the amount of UF that penetrated with LP or water into intact and damaged skin under the practical dose condition. When UF was applied to delipidized skin, the amount in the epidermis was approximately 50 and 25% that of intact skin after application with LP and water, respectively. Penetration of water-soluble drugs into delipidized skin has been reported to be higher than that into intact skin,\(^{3,14}\) which was not in case of intact skin. The UF amounts in the epidermis and dermis tended to be lower under the practical dose condition than under infinite conditions, but it was not significant. The effects of the application period and amount were the same with water as with LP (Fig. 2). In practical dose conditions, water should be evaporated from test material; however, UF was saturated in test material so that there was no difference because of the application amount. Thus, our further studies used the practical dose condition.

**REFERENCES**

1) Bekersky I, Fitzsimmons W, Tanase A, Maher RM, Hodosh E, Lawrence I. Nonclinical and early clinical development of tacrolimus ointment for the treatment of atopic dermatitis. *J. Am. Acad. Dermatol.*, 44 (Suppl.), S17–S27 (2001).

2) Kikuchi K, Tagami H. Comparison of the effects of daily applications between topical corticosteroid and tacrolimus ointments on normal skin; evaluation with noninvasive methods. *Dermatology*, 205, 378–382 (2002).

3) Gujar M, Banga AK. Vehicle influence on permeation through intact and compromised skin. *Int. J. Pharm.*, 472, 362–368 (2014).

4) Kubo H, Ohkuma N, Okawara A. Clinical effect of HF-264 ointment on atopic dermatitis. *Nishinokon J. Dermatol.*, 43, 261–263 (1981).

5) Kazama T, Ishibashi Y, Satomi I, Iwai M. Clinical evaluation of HF-264 ointment. *Nishinokon J. Dermatol.*, 43, 264–267 (1981).

6) Uchiyama M, Murakami J. Clinical experience of HF-264 ointment. *Nishinokon J. Dermatol.*, 43, 268–273 (1981).

7) Iju M, Takashima I, Kubota K. Clinical effect of HF-264 ointment on atopic dermatitis and napkin dermatitis. *Nishinokon J. Dermatol.*, 43, 256–260 (1981).

8) Sasaki R, Nakajima M, Takeuchi J. Clinical study of efficacy and safety of 5% ufenamate ointment and cream for eczematous skin changes (miliaria and diaper rash) in infants and children. *J. Pediatr. Dermatol.*, 16, 47–52 (1997).

9) Lavker RM, Dong G, Zheng PS, Murphy GF. Hairless micropig skin. A novel model for studies of cutaneous biology. *Am. J. Pathol.*, 138, 687–697 (1991).

10) Fujii M, Yamanouchi S, Hori N, Iwanaga N, Kawaguchi N, Matsu mato M. Evaluation of Yucatan micropig skin for use as an in vitro model for skin permeation study. *Biol. Pharm. Bull.*, 20, 249–254 (1997).

11) Imokawa G, Akasaki S, Hattori M, Yoshizuka N. Selective recovery
of deranged water-holding properties by stratum corneum lipids. *J. Invest. Dermatol.*, **87**, 758–761 (1986).

12) Kligman AM, Christophers E. Preparation of isolated sheets of human stratum corneum. *Arch. Dermatol.*, **88**, 702–705 (1963).

13) Takahara Y, Ohoshita M, Aratani T, Kudo S, Nishide K, Ito Y. Studies on the metabolism of butyl-2-[[3-(trifluoromethyl)phenyl]-amino]benzoate (HF-264) (4) percutaneous absorption in rats after dermal application. *Pharmacometrics*, **24**, 691–695 (1982).

14) Inoue K, Ogawa K, Suzuki Y, Okada J, Kusai A, Ikeda M, Nishimura K. The skin permeation mechanism of ketotifen: evaluation of permeation pathways and barrier components in the stratum corneum. *Drug Dev. Ind. Pharm.*, **26**, 45–53 (2000).

15) Smith EW, Surber C, Tassopoulos T, Maibach HI. Topical Dermatological Vehicles, *Topical Absorption of Dermatological Products*. (Bronaugh RL, Maibach HI eds.) Taylor & Francis, U.K., pp. 457–464 (2002).

16) Surber C, Smith EW. The mystical effects of dermatological vehicles. *Dermatology*, **210**, 157–168 (2005).

17) Higuchi T. Physical chemical analysis of percutaneous absorption process from creams and ointments. *J. Soc. Cosmet. Chem.*, **11**, 85–97 (1960).