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RESEARCH PAPER

Evaluation of Topical Application of Clobetasol 17-Propionate from Various Cream Bases

Jia-You Fang,[1,2] Kun-Long Shen,[1] Yaw-Bin Huang,[1] Pao-Chu Wu,[1] and Yi-Hung Tsai[1,*]

[1] *School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan*
[2] *School of Pharmacy, Chia Nan College of Pharmacy and Science, Tainan Hsien, Taiwan*

ABSTRACT

The effect of clobetasol 17-propionate (CP), a potent corticosteroid, in various cream bases on the permeation through artificial membrane was sought. Four formulations were then chosen for a further in vivo skin blanching assay. After calculation of the relationship between in vivo flux$_{0-8hr}$ determined from a surface recovery technique and in vitro release rate$_{0-8hr}$ of CP from various formulations, a high correlation coefficient of 0.9996 was achieved. Therefore, the in vitro release study could be used as an index to predict and evaluate the in vivo penetration capacity of CP cream to screen the effective formulation preclinically. After a series of in vivo investigations in this study, it was concluded that myristic acid-added formulations may show a bioequivalence with commercial Dermovate®. Furthermore, the flux calculated from the surface recovery technique and ΔE^* detected from the skin blanching assay may be useful as parameters evaluating the quality and effectiveness of CP cream.

Key Words: *Clobetasol 17-propionate; Topical application; Skin-blanching assay.*

INTRODUCTION

Clobetasol 17-propionate (CP) is widely considered to be the most potent of the currently available corticosteroids. The molecule itself possesses an unparalleled vasoconstrictive activity, over 1800 times than that of hydrocortisone (1,2). If different manufacturing concerns produce formulations containing CP in the same concentration, it may be expected that those preparations would show the same clinical efficiency as the origin of Dermo-

* To whom correspondence should be addressed.

vate® (Glaxo Co., UK). The terms bioequivalence and bioavailability have become commonplace in modern pharmaceutical research. More specifically, bioavailability of products refers to the release of steroids from the preparation followed by its penetration of the epidermis to produce the characteristic blanching response (3). The aim of our study is to evaluate the bioavailability of CP semisolid forms by both in vitro and in vivo assessments.

More recently, the effect that the composition of the formulation has on the extent of drug delivery has been researched to a much greater extent (4). Subsequently, not only the commercially available products were used in this study but also the original bases prepared from our laboratory according to USP and Japan patent. The bioengineering method of colorimetry is used in determining the skin blanching test in this study because it can act as a simple and accurate method for objectively measuring the blanching effects of corticosteroids (5). For the sake of evaluating the formulations selected from in vitro study, the noninvasive surface recovery technique for estimating the residual CP concentration remaining in the cream base after topical administration to human skin was also conducted (6,7).

MATERIALS AND METHODS

Materials

The following reagents were used: CP, *p*-phenylphenol, and Brij-35 (Sigma Chemical Co., St. Louis, MO); stearyl alcohol and cetyl alcohol (Merck, Darmstadt, Germany); Arlacel-C and croton oil (Tokyo Kasei Co., Tokyo, Japan); myristic acid (Wako Co., Tokyo, Japan); and liquid paraffin (Riedel-de Haen Co., Germany). The Dermovate cream was obtained from Glaxo (Pty.) Ltd. (Taiwan). Commercially available cream A and cream B were gifts from two pharmaceutical corporations of Taiwan. All other chemicals and solvents were of analytical grade.

Preparation of Cream Bases

The originally hydrophilic cream (USP) was composed of white vaselin (20.83%, w/w), stearyl alcohol (18.33%), propylene glycol (10%), and sodium laurylsulfate (1.67%), and purified water was added to make a total amount of 100%. The absorption cream (USP) was composed of white vaselin (33.33%), cetyl alcohol (15.00%), Arlacel-C (5.00%), and Brij-35 (0.50%), and purified water was added to make a total amount of 100%. The myristic acid-added base (Japan patent, 58-39616, 8 March 1983) was composed of white vaselin (14.40%), stearyl alcohol (1.90%), cetyl alcohol (2.90%), liquid paraffin (4.8%), Arlacel-C (0.95%), Brij-35 (0.95%), and myristic acid (3.80%), and purified water was added to make a total amount of 100%. The CP molecules were incorporated in the base to give a concentration of 0.05%.

Determination of Viscosity

The viscosity study was done in a cone and plate viscometer (model DV-2, Brookfield Co.). A total of 0.3 g of cream base was placed in the sample cup of the viscometer and allowed to stand for 1 hr to reach 25, 28, 32, 37, and 40°C. To obtain stable display readings, viscosity measurements were made after 90 sec.

In Vitro Release Rate Study

The artificial membrane was a lipophilic (FS) membrane (200 μm thickness, Millipore). This mesh filter was selected because it provided a large mean pore size of 3.0 μm for diffusion and showed a hydrophobic characteristic similar to that of the stratum corneum. The top of diffusion cell was covered with paraffin paper. The donor compartment of the cell was filled with 2 g of cream base containing CP. A total of 20 ml of 3:7 (v/v) ethanol-pH 7.4 McIlvaine buffer was used as the receptor medium. The available diffusion area of the cell was 2.54 cm^2. The cell was carried out at 37°C and the receptor phase was stirred by a magnetic stirrer at 700 rpm. At appropriate intervals, 500-μl aliquots of the receptor fluid were withdrawn and immediately replaced by an equal volume of fresh receptor solution to maintain a constant volume.

Subjects

Six healthy male volunteers aged from 24 to 27 years participated in this study. Informed consent was obtained from all subjects. None had any previous or existing history of skin disease. The laboratory temperature was kept in the range of 22–23°C, and the relative humidity was 60–65%. Disturbance in the laboratory during measurements was kept to a minimum.

Surface Recovery Technique

An accurately weighed 0.2 g of cream was spread uniformly over a sheet of cotton cloth (22 cm^2) for an 8-hr administration period by the occlusive dressing technique

(ODT) (8). These pieces of cloth were then applied on both volar forearms of volunteers (four pieces on each forearm). The cloth was then covered with surgical adhesive tape (Micropore®, 3M). After recovering, the residual cream base remaining on the skin surface was withdrawn by a sterile cottonwool swab immersed in methanol solution. The difference between applied and recovered amounts corresponded to the cumulative absorbed amount.

Determination of CP in Cream Base

For the surface recovery technique, one cotton cloth and one cottonwool swab containing cream base were mixed with 5 ml of methanol solution containing 2 μg/ml *p*-phenylphenol as an internal standard in a glass-stoppered centrifuge tube and then followed by mechanical shaking for 30 min. After centrifugation for 10 min at 3000 rpm, the supernatant organic layer was directly injected into the HPLC. The recoveries of CP from Dermovate, cream A, cream B, and myristic acid-added base were 75.54 ± 3.08%, 80.66 ± 4.79%, 76.16 ± 5.18%, and 89.74 ± 4.05%, respectively.

Skin Blanching Assay by Colorimetry

The method of colorimetry was modified from Fang et al. (9). A colorimeter (Chroma Meter- CR221, Minolta, Japan) was used as a measure of color difference. The instrument records color reflectance three dimensionally (L*, a*, b*). Differences in color between treated site and the control site were expressed as ΔL*, Δa*, Δb* and $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Skin blanching was evaluated at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, 29, 34, and 48 hr after the CP cream was applied. The product was removed 0.5, 1, 4, and 8 hr after application. The colorimetry results were expressed as differences from the control, which is the value obtained from one adjacent untreated site. Thereafter, changes in color on the treated sites were determined against the subject's own baseline standard.

HPLC Analytical Method

The CP content of the various samples was analyzed by an HPLC system (Jasco Co., Japan). A 12.5-cm-long, 4.0-mm inner diameter, stainless steel column with a Lichrospher C-18 column (Merck) was used. An automated integrator system (model 855-AS, Jasco) was used to determine the area under the curve. The drug sample was mixed with a suitable amount of *p*-phenylphenol as an internal standard. The mobile phase for CP consisted of 65% methanol and 35% pH 3.5 aqueous phase adjusted by 0.05% acetic acid. The column effluent was passed through the UV detector set at a wavelength of 240 nm with a flow rate of 1.1 ml/min. The retention times of *p*-phenylphenol and CP were found to be 4.4 and 9.0 min, respectively.

RESULTS AND DISCUSSION

Viscosity of CP Cream Bases

The viscosity showed a significant difference among various cream bases as observed in Table 1. The viscosity determined by a viscometer decreased after the increase of detected temperature. The viscosity of cream A was significantly lower than that of the others at all temperatures. Hydrophilic and absorption formulations revealed high viscosity at lower temperatures; nevertheless, the value reduced largely as enhancing the detected temperature. On the other hand, the fact that the near-constant

Table 1

Viscosity and Activation Energy Measured from Arrhenius Equation of Various Clobetasol 17-Propionate Cream Bases

Formulation	Viscosity (×1000 cps) 25°C	28°C	32°C	37°C	40°C	Activation Energy (kJ/mol)	Correlation Coefficient (*r*)
Dermovate®	131.33 ± 9.81	110.87 ± 13.81	84.87 ± 2.78	76.07 ± 10.41	69.33 ± 5.60	32.7	0.9795
Cream A	16.63 ± 1.15	13.23 ± 2.54	9.53 ± 1.45	7.35 ± 0.83	1.74 ± 0.04	99.2	0.8925
Cream B	113.00 ± 5.35	112.33 ± 29.85	99.87 ± 0.98	91.40 ± 14.06	86.47 ± 22.59	14.8	0.9863
Hydrophilic	181.67 ± 10.50	128.64 ± 11.33	106.33 ± 14.50	76.37 ± 5.88	59.60 ± 0.43	54.3	0.9935
Absorption	154.67 ± 11.15	132.33 ± 19.69	129.33 ± 8.06	97.93 ± 2.74	52.13 ± 0.84	48.4	0.8969
Myristic acid	111.33 ± 8.22	99.53 ± 3.11	75.93 ± 2.25	67.80 ± 5.38	27.97 ± 10.62	36.8	0.8955

Values are means ± SD, $n = 3$.

viscosity of base could be achieved in cream B at various temperatures demonstrated that it can bear unfavorable conditions during long-term storage. For further exploration of the relationship between viscosity and temperature, the activation energy (E_a) was treated according to the Arrhenius equation to obtain the thermodynamic parameters of formulations as shown in Table 1 (10). The E_a was quite different in various formulations, possibly resulting from the different components or proportions of additives. The highest and lowest E_a was observed in cream A and cream B, respectively. Moreover, the E_a of the myristic acid-added formulation approximated that of Dermovate more than the others did. Accordingly, the E_a value can be used to evaluate the discrimination of composition among various formulations so as to be an index of quality of CP cream.

In Vitro Release of CP Through Artificial Membrane from Cream Bases

The release rate of CP from various cream bases can be distinguished significantly by using the FS artificial membrane as shown in Fig. 1. After the comparison of release rates of shorter duration (0–8 hr) and longer duration (0–48 hr) of each formulation, it demonstrated that CP was slowly released from the cream bases at the beginning. However, the trend of both release rates in various bases were in accordance with each other. The highest and lowest release rates were observed in cream A and cream B, respectively, accorded with the result of E_a calculated from viscosity. In addition, the release rate of myristic acid-added cream was also similar to that of Dermovate. Subsequently, this result found that the viscosity played a very important role on the release rate of the drug from cream (11,12). The semisolid base with lower viscosity can easily and quickly spread on stratum corneum because contacting with skin surface results in the high release rate of CP. Therefore, viscosity can be an important parameter in anticipating the release rate of CP from creams. Furthermore, according to the data of viscosity, E_a, and release rate, the equivalence of quality observed between Dermovate and myristic acid- added base suggested the possibility of bioequivalence between these two formulations.

Surface Recovery Technique

The base prepared from our laboratory chosen from the in vitro evaluations was myristic acid-added cream (Japan patent, 58-39616, 8 March 1983) because of the highest flux and CP residual in skin among the USP and patent formulations. The results from using the surface

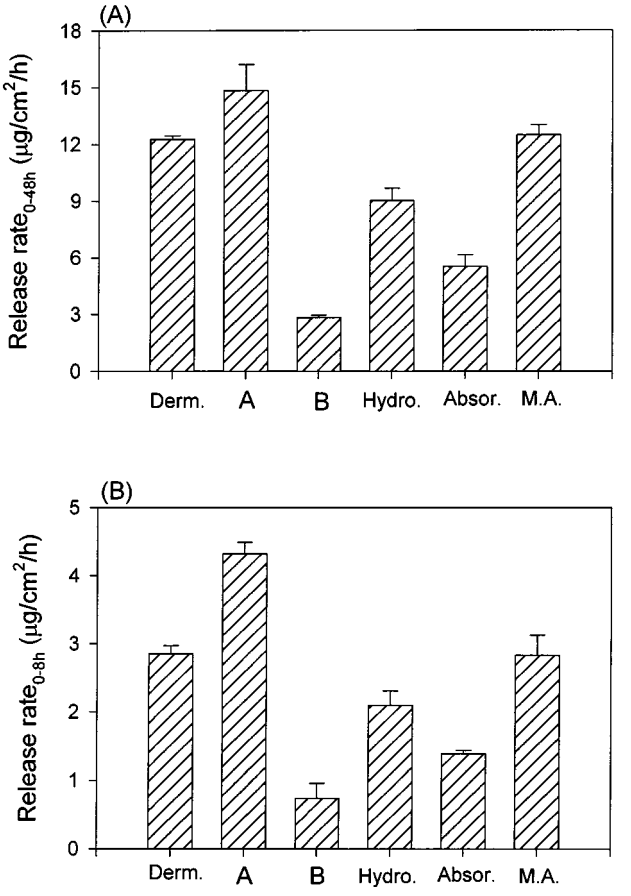

Figure 1. The release rate of CP from various cream bases through an artificial membrane. Derm., Dermovate; Hydro., hydrophilic cream; Absor., absorption cream; M.A., myristic acid-added base. Each value represents the mean ± SD ($n = 3$).

recovery technique and the cumulative absorbed amount of CP, which are the differences between applied and recovered amounts, are shown in Fig. 2. The applied amount was determined as the recovered amount in 0 hr, which was calculated by the drug concentration from the cream base applied on the skin and immediately peeled off. The cumulative absorbed amount–time profile fitted well with the zero-order equation. It was found that the in vivo $flux_{0-8hr}$ retained in and through human skin increased in the order of cream B < myristic acid- added base < Dermovate < cream A. After calculation of the relationship between in vivo $flux_{0-8hr}$ and in vitro release $rate_{0-8hr}$ of CP from four formulations, a high correlation coefficient of 0.9996 was obtained. This result suggested that the process of CP from cream base to skin reservoir played a principal role on the topical application to human skin. There was no significant difference (*t*-test, p

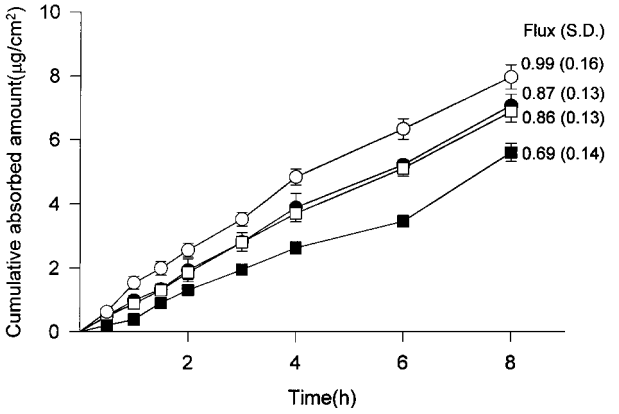

Figure 2. Cumulative absorbed amounts versus time profiles of CP after topical administration of various cream bases in humans. Dermovate (●), cream A (○), cream B (■), myristic acid-added base (□). Each value represents the mean ± SD ($n = 6$).

> 0.05) in CP $\text{flux}_{0-8\text{hr}}$ between Dermovate and myristic acid- added base, indicating topical bioequivalence could be achieved when considering the result of the surface recovery technique.

Skin Blanching Assay by Colorimetry

The advantages of the blanching assay are that it is noninvasive, inexpensive, reproducible, and safe (13). In clinical circumstances, when the degree of sensitivity of the skin to blanching is required, one can simply estimate the degree of blanching while comparing it to normal neighboring skin as the value of color difference (ΔL*, Δa*, Δb*). After the application of CP creams for 1 hr and then removal as shown in Fig. 3, skin pallor became lighter and its redness faded. This phenomenon corresponded to an increase in L* and a decrease in Δa*. Therefore, the intensity of blanching induced by CP varies directly with L* coordinate and inversely with Δa* coordinate. Measurements on the b* revealed no significant changes, perhaps because this value showed lower sensitivity to skin color change induced by blanching than ΔL* and Δa* values. This result was the same as the skin color change induced by erythema after topical application of capsaicin, indicating b* was less sensitive (9). Accordingly, coordinates ΔL* and Δa* were found to be more discriminating than Δb* for the application of CP creams.

The intensity of ΔL* increases with time to reach a peak at 6 or 8 hr before leveling off for four cream bases as shown in Fig. 3(A). The curves of Dermovate, cream

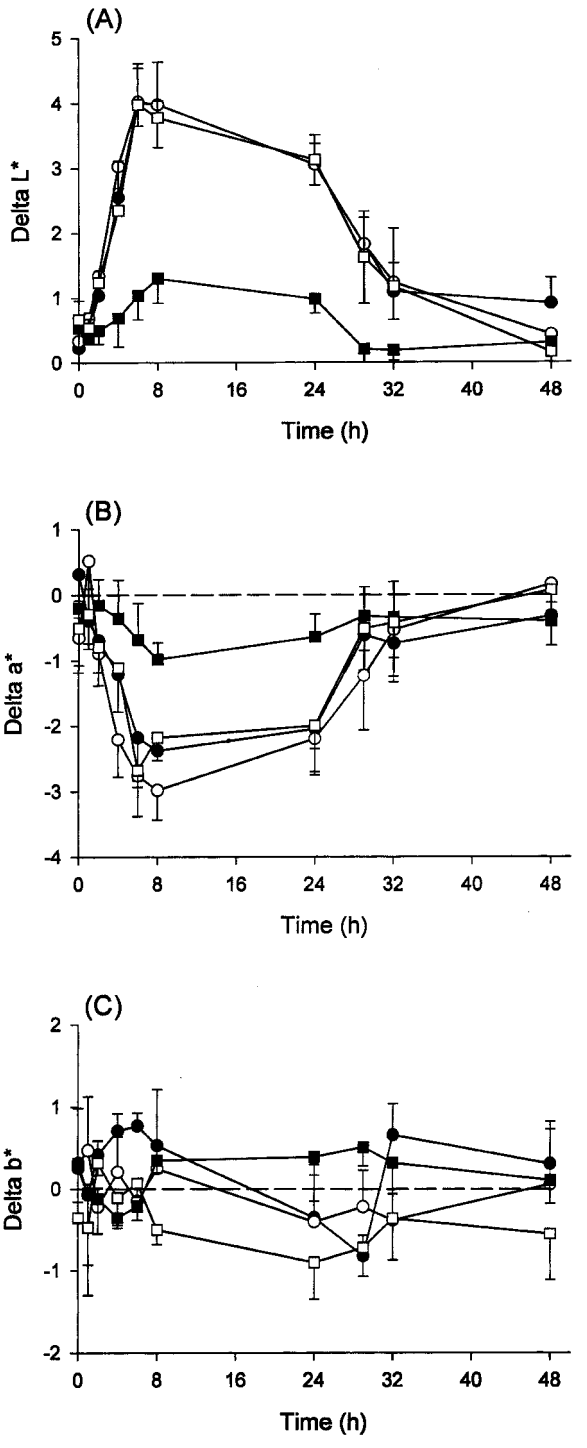

Figure 3. Color differences measured by ΔL* (A), Δa* (B), and Δb* (C) of colorimetry between treated sites and neighboring untreated sites for CP from various cream bases after topical administration for 1 hr in humans. Dermovate (●), cream A (○), cream B (■), myristic acid-added base (□). Each value represents the mean ± SD ($n = 6$).

A, and myristic acid-added base were approximate, except the data points at 48 hr. There was no significant difference observed between the data points of 0 and 48 hr after application for ΔL* values of cream A, cream B, and myristic acid-added base, suggesting that skin blanching faded to a normal dermal condition after 48 hr with a 1-hr administration of CP. On the other hand, Dermovate maintains skin blanching for more than 48 hr, as shown in Fig. 3(A). The ΔL* values of cream B were significantly lower than those of the other formulations, possibly because of the high viscosity and low release rate of CP for cream B. This result was consistent with that of the surface recovery technique, which showed that cream B had the lowest flux among four formulations. The behavior of Δa* during skin blanching is opposite to that of the ΔL* value, as shown in Fig. 3(B). The a* coordinate started at a high level and decreased, returning to the initial level over 32 hr. Accordingly, Δa* may reflect shorter vasoconstriction levels than ΔL*, which returned to the initial level at 48 hr. The skin blanching did not reveal remarkable changes for cream B in light of Δa*, consistent with the result of ΔL*. The parameter Δa* is well suited to assess the amount of blood in subepidermal vascular plexus and papillary loops (14), demonstrating that CP develops its function on both of these parts of skin. The Δb* coordinate changes are variable in Fig. 3(C); however, there seems to be trends for both cream B and myristic acid-added base that show positive and negative Δb* values after application, respectively.

To indicate skin color change by the multiple index, $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ was calculated because the ''true'' color of the skin should be expressed as an admixture of the a*, b*, and L* values (15). The ΔE* changes induced by CP from four formulations are shown in Fig. 4. Maximum blanching was observed 6 or 8 hr after application of CP creams, concurring with the profile of ΔL*. Similar to the results of the surface recovery technique, ΔE* coordinate still exhibited the ranking of the skin blanching potency increased in the order of cream B < myristic acid-added base < Dermovate < cream A.

Comparison of the Skin Blanching Assay for Various Administration Durations

The ΔE* values of four CP cream bases for various administration durations after the time of application are shown in Table 2. Although the ΔE* values increased after the elongation of administration duration in the same time plot, the differences of ΔE* among various durations were not evident and thorough. This is because the glucocorticoid receptors in the dermal tissues may be fully saturated within 1–1.5 hr of topical dosing with

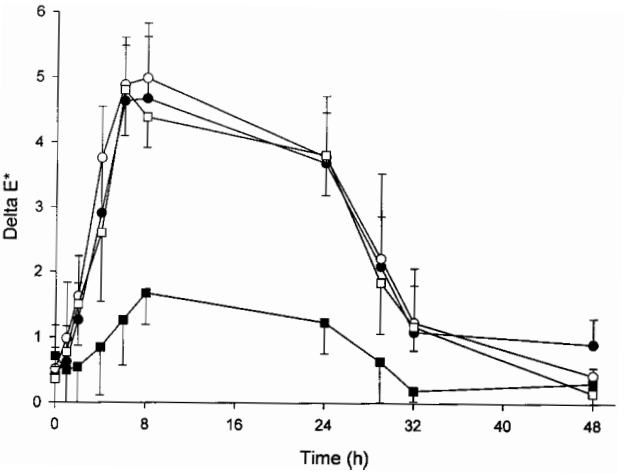

Figure 4. Color differences measured by ΔE* of colorimetry between treated sites and neighboring untreated sites for CP from various cream bases after topical administration for 1 hr in humans. Dermovate (●), cream A (○), cream B (■), myristic acid-added base (□). Each value represents the mean ± SD ($n = 6$).

corticosteroids because CP is a potent steroid to attach the receptors (16). Furthermore, the fact that CP has less polarity resulted in partitioning into the hydrophobic stratum corneum easier than the other corticosteroids did (17). The administration duration of 0.5 hr may be enough to occupy glucocorticoid receptors completely and to produce effectively pharmacological activity. In the surface recovery technique, the cumulative absorbed amount of CP corresponded to the combination of drug remaining in the skin reservoir and passing through skin. Although the cumulative absorbed amount of CP increased with increasing administration duration at a constant zero-order flux from 0 to 8 hr, it could be expected that most CP molecules transferred into the systemic circulation directly at a later period.

For comparing the potency of skin blanching of various formulations and administration durations by the summary parameters instead of multipoint comparisons, area under the curve (AUC) and the sum of ΔE* (∑ΔE*), calculated from Table 2, are determined as shown in Table 3. The AUC analysis, similar to that after systemic administration, accounts for both the intensity and the duration of action of the pharmacodynamic response. Nevertheless, the calculation of AUC is statistically debatable because of the lack of nocturnal values (5). Accordingly, the algebraic sums of mean adjusted values (∑ΔE*) were selected as the other index. After the comparison of summary parameters by various administration durations, 0.5 hr of dosing may act as a maximum dura-

Table 2

The ΔE Values of Colorimetry for Various Administration Durations of Clobetasol 17-Propionate from Various Cream Bases*

Formulation	Administration Duration (hr)	ΔE* After the Time of Administration								
		1 hr	2 hr	4 hr	6 hr	8 hr	24 hr	29 hr	32 hr	48 hr
Dermovate®	0.5	0.39	1.37	2.95	4.58	4.42	3.64	2.21	1.38	0.82
	1	0.62	1.26	2.91	4.63	4.67	3.69	2.09	1.09	0.91
	4	—	—	3.32	5.04	4.95	3.66	2.05	1.82	0.90
	8	—	—	—	—	4.65	4.16	2.67	2.07	1.17
Cream A	0.5	0.62	1.52	3.84	4.92	5.07	3.92	2.21	1.14	0.66
	1	0.98	1.62	3.76	4.88	4.98	3.78	2.21	1.23	0.43
	4	—	—	4.13	5.02	5.10	3.77	2.60	2.08	0.98
	8	—	—	—	—	5.19	4.44	2.84	1.94	1.47
Cream B	0.5	0.45	0.50	0.78	1.31	1.51	1.12	0.44	0.16	0.26
	1	0.49	0.54	0.85	1.26	1.67	1.23	0.64	0.19	0.31
	4	—	—	1.87	2.35	2.97	2.20	1.35	0.43	0.38
	8	—	—	—	—	3.90	3.01	2.04	1.11	0.52
Myristic acid	0.5	0.88	1.61	2.86	4.77	4.50	3.54	2.07	1.04	0.22
	1	0.77	1.50	2.60	4.80	4.39	3.81	1.84	1.97	0.16
	4	—	—	3.10	4.94	4.88	3.47	1.95	1.03	0.95
	8	—	—	—	—	4.23	3.38	2.34	1.45	1.09

Values are means ± SD, $n = 6$.

tion and may be enough to achieve the effective potency of clinical therapy because the higher durations may restrict their potencies to the level of 0.5 hr. The AUC_{total} of cream B increased after the increase of administration duration. This phenomenon is possibly due to the high viscosity and low release rate of CP from cream B, and the time for saturating the receptors in skin should be longer for cream B than those for the others.

The rank order of four cream bases was similarly based on either AUC or $\sum\Delta E^*$ values. The skin blanching potency of cream A was higher than those of the other formulations because of the higher release rate of CP from cream A than the others, although there was no significant difference among the values of Dermovate, cream A, and myristic acid-added base. In light of the four summary parameters shown in Table 2, the skin blanching potency of myristic acid-added base approaches that of Dermovate after comparing the four formulations thoroughly. The bioequivalence was obtained between these two formulations because the vasoconstriction assay can predict the clinical effectiveness of a given formulation (18).

In fact, the intensities of skin blanching of formulations used in this study were close, except cream B. In addition to the fact that CP reveals a potent capability for clinical therapy is the use of ODT. Hydration results from water diffusing from underlying epidermal layers or from the accumulation of perspiration after occlusion. In addition, hydration opens up the compact substance of the stratum corneum, and both increase the rate of penetration and the possibility of formation of a reservoir for corticosteroid (2). The occlusion of skin surface may result in an almost 100-fold difference in corticosteroid absorption over simple topical application (19). Temperature increase may also play a role in increasing penetration after occlusion. Subsequently, the characteristic of the vehicle itself was negligible due to the effect of drug and ODT used in this present blanching assay when considering these formulations, except cream B.

CONCLUSIONS

After calculation of the relationship between in vivo $flux_{0-8hr}$ measured from the surface recovery technique and in vitro $release\ rate_{0-8hr}$ of CP from four formulations, a high correlation coefficient of 0.9996 was obtained, which suggested that the process of CP from cream base to skin reservoir played an important role on topical application to human skin. The skin blanching assay of CP from creams detected by colorimeter showed a phenomenon corresponded to an increase in ΔL* and a decrease in Δa*. Measurement on the Δb* value revealed no significant changes. The intensities of skin blanching of various cream bases were approximate, except cream B. Besides the reason of CP behaving as a potent capability in clinical therapy, an-

Table 3

The AUC and ∑ΔE of Colorimetry for Various Administration Durations of Clobetasol 17-Propionate from Various Cream Bases*

Formulation	Administration Duration (hr)	AUC_{0-8hr}	AUC_{total}	$\sum\Delta E^*_{0-8hr}$	$\sum\Delta E^*_{total}$
Dermovate®	0.5	21.93 ± 3.19	124.02 ± 27.06	13.71 ± 2.79	21.76 ± 3.38
	1	22.26 ± 5.49	124.36 ± 32.10	14.09 ± 3.77	21.87 ± 4.97
	4	—	129.07 ± 18.12	—	21.74 ± 3.76
	8	—	120.59 ± 36.50	—	14.72 ± 4.28
Cream A	0.5	25.49 ± 7.73	132.16 ± 31.10	15.97 ± 4.55	23.90 ± 5.57
	1	25.67 ± 5.25	129.17 ± 26.89	16.22 ± 3.14	23.87 ± 4.79
	4	—	137.66 ± 31.18	—	23.68 ± 6.01
	8	—	129.69 ± 25.27	—	15.88 ± 3.34
Cream B	0.5	6.89 ± 2.12	36.09 ± 11.76	6.53 ± 2.51	8.51 ± 2.95
	1	7.19 ± 2.26	40.31 ± 15.86	4.81 ± 1.51	7.18 ± 3.08
	4	—	68.93 ± 17.12	—	11.55 ± 3.27
	8	—	85.83 ± 26.36	—	10.63 ± 3.30
Myristic acid	0.5	23.06 ± 4.66	116.15 ± 21.88	14.62 ± 2.61	21.49 ± 3.53
	1	22.21 ± 3.29	117.09 ± 13.34	14.06 ± 2.05	21.04 ± 3.02
	4	—	118.52 ± 25.51	—	20.32 ± 4.16
	8	—	101.19 ± 27.89	—	12.49 ± 3.22

Values are means ± SD, $n = 6$.

other one is the use of ODT during the experiment, causing the hydration of stratum corneum and dermis. The myristic acid-added base prepared in our laboratory showed a bioequivalence with Dermovate, according to the results of this study, and include anti-inflammatory effects in rats and surface recovery technique and skin blanching assay in humans. Furthermore, the flux calculated from the surface recovery technique and ΔE* detected from the skin blanching assay may be useful as parameters to evaluate the quality and effectiveness of CP cream.

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