Circadian Activity of Topical 0.05% Betamethasone Dipropionate in Human Skin *In Vivo*

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The influence of treatment duration, vehicle, and time of day of application on topical 0.05% betamethasone dipropionate uptake into human stratum corneum and the resulting skin-blanching response was investigated in human subjects. Drug uptake into stratum corneum and the resulting skin color changes measured with a chromameter demonstrate an equilibrium delay. Maximal drug uptake occurred at 2 h, whereas maximal skin color changes occurred 6 h after a single application. Extent of decreased skin color was dependent on vehicle, treatment duration, and time of day of application. Time of maximal decreased skin color occurred at midnight independent of vehicle, treatment duration, or time of day of application. This time of maximal drug activity coincides with the well-known time period of lowest circulating cortisol concentrations (2000–0400 h). Application of a single 2- or 6-h dose of the 0.05% cream at 1600 h produced more extensive and prolonged changes in skin color over 24 h than a 0900-h application in the same subject. These data demonstrate that the extent and duration of topical corticosteroid activity in human skin is influenced by vehicle, treatment duration, and time of day of application. The prolonged changes in skin color measured with a single dose applied at 1600 h suggest that a once-a-day dosing regimen in the late afternoon may be sufficient for dermatologic therapy. Elucidation of these circadian responses with topical corticosteroids may provide a rational basis for the future re-evaluation of the appropriate therapeutic regimen with this class of drugs in dermatologic medicine. *Key words: human/in vivo/skin/chronobiology/corticosteroid. J Invest Dermatol 102:734–739, 1994*

Topical corticosteroids are synthetic analogs of cortisol, a naturally occurring chemical in the body produced primarily in the adrenal gland, which influences carbohydrate, protein and lipid metabolism, and electrolyte and water balance [1]. Topical corticosteroids also prevent or suppress the development of the erythema and edema in the inflammatory response and hyperproliferation, which is the basis for their widespread use in dermatologic medicine. The ability of topical corticosteroids to produce a skin-blanching bioresponse in the skin following topical application, the so-called "skin-blanching response" [2], has been demonstrated to correlate with their efficacy in treating the inflammatory and hyperproliferative disease psoriasis [3], and has been used to rank in order the potency of various synthetic corticosteroids in a variety of vehicles, both innovator and generic products [4]. The pharmacodynamic response of skin blanching is influenced by the corticosteroid structure [5,6], the vehicle formulation [4,7–9], and the concentration of the steroid applied [10,11].* Most published evaluations of corticosteroid formulation pharmacodynamic activity have used the McKenzie-Stoughton vasoconstrictor assay [2], in which a single dermatologic dose of a drug formulation (1- to 5-mg formulation/cm² skin) is applied to the ventral forearm of human subjects for 16 h, removed, and the resulting skin-blanching response at the treated skin site assessed 2 h later. This approach does not identify the maximal response, the time of maximal response, or the mechanistic basis for the lack of a response. Identification of the maximal response would require measuring the response at multiple times over a 24-h period. The merits of monitoring the profile of the skin-blanching response periodically over 1–2 d have been discussed previously [8,13]. Further, measuring the skin-blanching response visually is subjective, varying among investigators, environments, and subject populations. Objective measure of changes in skin color can be performed with a chromameter, which measures skin color noninvasively on three scales [11,12,14]: luminosity (L scale) measures reflected white light, a scale measures reflected light in the green-red (low-to-high values) light spectrum, and b scale measures reflected light in the blue-yellow (low-to-high values) light spectrum. Changes in the reflected red-green color scale on the a scale on the chromameter have been shown previously to inversely correlate with the visual assessment of the skin-blanching response with various drug concentrations [12],* vehicle formulations,* durations of drug application [12], and potencies of corticosteroids [14].

A mechanistic understanding of the lack of a skin response can be investigated by quantitating the drug uptake into the rate-limiting barrier to percutaneous absorption, the stratum corneum. Lack of drug uptake into this skin layer would explain the lack of a skin response. Quantitation of drug uptake of topical corticosteroids in human skin *in vivo* has been previously demonstrated for betamethasone dipropionate using a validated skin-stripping method [8,12,13].

The present study was performed with 0.05% betamethasone dipropionate in a variety of vehicle formulations using five dura-

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Abbreviations: DLC, diprolene AF cream; DLO, diprolene augmented ointment; DSC, diprosone cream; DSL, diprosone lotion; DSO, diprosone ointment.

* Pershing LK, Bakhtian S, Poncelet CE, Corlett JL, Shah VP: Disparity between the pharmacokinetic and pharmacodynamic dose response of triamcinolone acetonide cream formulations in humans (abstr.). *J Invest Dermatol* 100:591, 1993.

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tions of treatments to identify the maximal skin-color response, the time of the maximal response, and duration of that response over 24 h using both subjective and objective measurements. Skin stripping of the treated skin sites was also performed to determine whether the skin-blanching responses were related to differences in drug uptake into the stratum corneum.

MATERIALS AND METHODS

Drug Formulations All of the 0.05% betamethasone dipropionate formulations—Diprolene-augmented ointment (DLO), Diprolene AF cream (DLC), Diprosone ointment (DSO), Diprosone cream (DSC), and Diprosone lotion (DSL)—were used as purchased from the University of Utah Hospital Pharmacy. Micropore surgical paper tape (3M, St. Paul, MN) and the 2.2-cm diameter rubber O-rings purchased from a local plumbing supply store were used to produce the protective unoccluding tapeguards [12]. The chromameter (Minolta CR200) used to objectively and noninvasively assess the skin-blanching response *in vivo* was purchased from the Minolta Corp. (Industrial Meters Division, Ramsey, NJ).

Human Subjects Human subjects utilized in the following studies included equal numbers of healthy Caucasians, male and female, all between 19 and 45 years of age, currently without any chronic topical drug therapy or acute topical drug therapy within 1 month of the study. Subjects were not selected based on skin type or chromameter assessment of skin color. Visual assessment of skin color did not change significantly, however, over the 24-h experimental time period. Subjects were remunerated for their participation. All protocols were approved by the University of Utah Institutional Review Board. The same human subjects were not utilized in every study, but many of the subjects were tested on more than one occasion, with a minimum of 1 month between studies.

Drug Application Ten microliters of commercial drug formulation was routinely applied to a 3.8-cm^2 area of forearm skin *via* a 1-ml tuberculin syringe. The topical formulation was spread over the skin surface area with the smooth end of a 1.5-ml polypropylene conical microcentrifuge tube to produce a typical dermatologic dose of 2.6 $\mu l/cm^2$ skin, equivalent to a relative film thickness of 26 μm. Multiple sites on a forearm were treated, the distance between sites being 3 cm center to center, all sites demarked to an area of the forearm that was a minimum of 3 cm above the wrist and 3 cm below the antecubital fossa. Within this treatment area, a maximum of five sites per forearm were possible. All treated skin sites remained unoccluded, but were protected from the environment with a tapeguard made in our laboratory [12] using paper tape (Micropore) cut into a 16-cm long × 5.4-cm wide length and a 5.4 × 5.4 cm length, a 2.2-cm inside diameter rubber O-ring (3-mm height), purchased from a local plumbing store, and a 3.5 cm × 3.5 cm piece of single layer of open weave gauze (Johnson and Johnson) for each tapeguard.

Assembly of the tapeguard requires generating a 2-cm diameter hole in the middle of the long and short lengths of the paper tape with a cork borer. To the adhesive side of the longer piece of paper tape, a single layer of gauze is attached. The O-ring is subsequently centered over the gauze and paper tape and held in place with the remaining smaller piece of paper tape. In this tapeguard the O-ring serves as a spacer between the treated skin and the top of the tapeguard, allowing air circulation to the treated skin site and thus providing a nonoccluded environment for drug treatment. The open weave gauze protects the treated skin site from the environment yet maintains a nonoccluding skin state. The tapeguards are prepared 24 h in advance of a study and stored on wax paper for easy removal. One tapeguard is prepared for each skin site to be treated. Each tapeguard is placed such that the O-ring is centered over the treated skin site.

Drug Removal At the end of the treatment duration, residual drug was removed with three independent dry cotton applicators and left uninterrupted for 3–5 min before tape-stripping the treated skin site or pharmacodynamic assessment.

Drug Uptake Quantification Treated skin sites were tape-stripped with Transpore surgical tape (3M) using a method previously described [12]. Briefly, 11 1.4-cm-diameter tapediscs were generated per skin site from a roll of the surgical tape with a cork borer. The first tapedisc was applied to the center of the 2-cm-diameter treated skin site with a forceps. After applying firm pressure to the tapedisc surface with the blunt end of the forceps four times, the first tapedisc was carefully removed from the skin with the forceps and discarded. The same procedure was repeated with the subsequent 10 tapediscs at the same skin site and combined into a prelabled 1.5-ml polypropylene capped conical microcentrifuge tube and stored at −70°C until analyzed for drug content by high-performance liquid chromatography HPLC. This tape-stripping method consistently (mean coefficient of variation = 24%) removes 107 ± 36 μg stratum corneum per cm^2 surface area of the tapedisc [8]. Data are presented as amounts of betamethasone dipropionate per surface area of the tapedisc or $\mu g/cm^2$.

Skin-Blanching Response All pharmacodynamic responses to drug treatment were measured noninvasively both subjectively using a 4-point (0–4, low–high) visual assessment scale [8] and objectively with the Minolta CR200 chromameter [12] periodically over a 24-h period.

Experimental Design

Pharmacokinetic and Pharmacodynamic Dose Response: Five different treatment durations of 0.05% DLC were applied to each forearm of six human subjects at 1600 h in the month of February. The locations of the treatment durations on both left versus right ventral forearms were randomized in each subject. The visual skin-blanching response was assessed by two independent investigators unaware of the type of drug treatment. The influence of treatment duration (0.5, 2, 6, or 16 h) on drug uptake was assessed at the end of the treatment duration only. The resulting pharmacodynamic response to each drug treatment was measured periodically over the following 24 h (0.5, 2, 6, 8, 16, 20, and 24 h after drug application).

Influence of Vehicle on Skin-Blanching Response: The five drug formulations were applied to the ventral forearm skin of 10 subjects in a randomized design with respect to location on the arm and left versus right arm at 1600 h in August, 6 months prior to the dose-response study. The five formulations represented two ointment formulations, two cream formulations, and one lotion. These formulations differ in their potency according to Stoughton [4]: Diprolene augmented ointment (class I) > Diprolene AF cream = Diprosone ointment (class II) > Diprosone cream (class III) > Diprosone lotion (class V). A 6-h unoccluded treatment duration was used for all treated skin sites on both forearms. One forearm was used to monitor the pharmacodynamic skin-blanching response over time whereas the other forearm was used for quantitation of drug uptake into the stratum corneum at the end of the treatment duration using a skin-stripping method developed and validated in our laboratory [8,12]. The same randomization of treatment durations used for the forearm receiving the pharmacodynamic treatment was used for the other forearm to be tape-stripped. The visual skin-blanching response was assessed by two independent investigators blinded to the drug treatments. Another blinded investigator measured the objective skin response with the Minolta CR200 chromameter, while yet another blinded investigator tape-stripped the treated skin sites. Visual and chromameter assessments of the blanching response were measured at untreated and drug-treated sites at 6, 8, 16, 18, 20, and 24 h after drug application.

Influence of Time of Day of Application on Skin-Blanching Response: Five different treatment durations (0, 0.25, 0.5, 1, 2, and 6 h) of 0.05% DLC, a class II potency topical corticosteroid, were applied unoccluded to each forearm of six healthy human subjects, of which 50% were female. The location of the treatment durations on the left versus right forearm and time of day of application were randomized in each subject. This study was performed in August, 6 months after the dose response study. The drug formulation was applied to all sites on one forearm at 0900 h and the other forearm of the same subject at 1600 h. Pharmacodynamic measurements were measured at the same time of the day for both drug application times. In this way, the influence of treatment duration (0.25, 0.5, 1, 2, and 6 h) on the time to maximal skin-blanching response over the following 24 h could be measured as a function of when the drug treatment was applied. The area under the chromameter a scale time curve was determined using the trapezoidal rule [15]:

$$\Sigma \{0.5 \times [(\text{time}_{x+n} - \text{time}_x) \times (\text{a scale}_{x+n} + \text{a scale}_x)].$$

HPLC Analysis Skin strippings collected from the drug-treated sites were analyzed for drug content (betamethasone dipropionate) according to an HPLC method developed in our laboratory as previously described [8]. Drug content extracted from the skin strippings was quantitated against extracted tapediscs (10 1.4-cm-diameter Transpore tapediscs) spiked with known drug concentrations. Extraction efficiency of the skin strippings was >95%. Sensitivity limits for betamethasone dipropionate detection was 2 ng on column or 50 ng/ml.

Statistical Analysis Data were evaluated for statistically significant differences ($p < 0.05$) using the nonparametric Wilcoxon Signed Rank test and one-way repeated measures analysis of variance (ANOVA) using STATVIEW II software (Calabassas, CA).

RESULTS AND DISCUSSION

Pharmacokinetic/Pharmacodynamic Dose Response The relationship between drug uptake as a function of treatment dura-

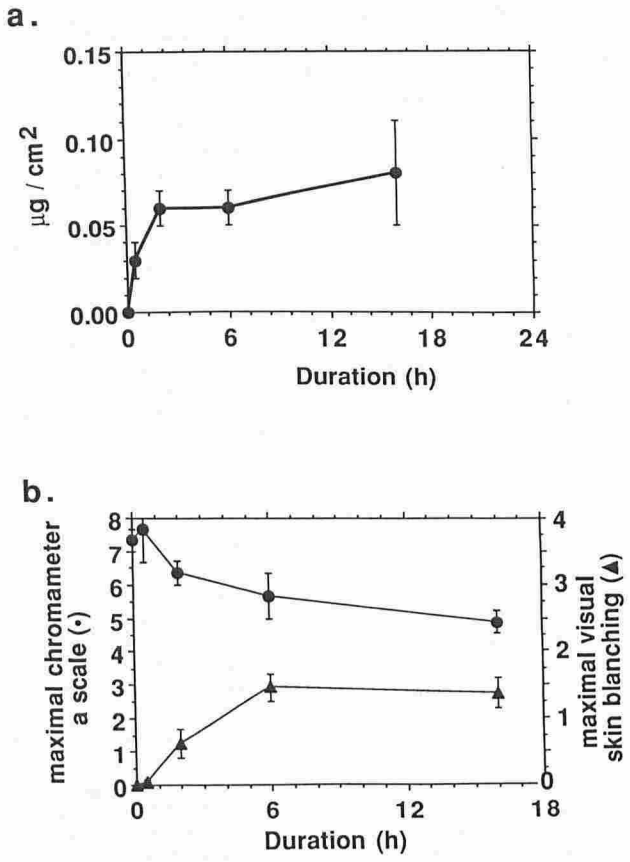

Figure 1. *a)* Uptake of betamethasone dipropionate into human stratum corneum *in vivo*. Drug uptake into ventral forearm stratum corneum was determined by HPLC and presented as micrograms of drug per surface area (cm^2) of the tapedisc following treatment with 0.05% DLC for 0.5, 2, 6, and 16 h. Mean ± SEM, n = 6 subjects. *b)* Visual skin-blanching responses and chromameter a scale skin color changes in human skin treated with 0.05% DLC. Subjective visual and objective chromameter a scale assessment of skin color following unoccluded treatment with 0.05% DLC for 0.5, 2, 6, and 16 h. *Solid triangle,* visual assessment of the skin-blanching response; *solid circle,* objective chromameter a scale values. Mean ± SEM, n = 6 subjects.

tion with 0.05% DLC is shown in Fig 1*a*. Increasing the treatment duration increases drug uptake ($\mu g/cm^2$) concentration in the stratum corneum up to 2 h. Increasing the treatment duration further to 6 or 16 h did not further increase the drug uptake into human stratum corneum.

Plotting the visual skin-blanching response and the chromameter a scale values measured after drug removal as a function of treatment duration demonstrates that increasing the treatment duration decreased chromameter a scale skin color and increased visual skin blanching to the greatest extent with 6 h of treatment (Fig 1*b*). The skin-blanching response to the 0.05% DLC measured with the chromameter a scale inversely correlated with those assessed visually at all time points of measurement ($r = -0.8$).

The maximal pharmacodynamic response was not achieved with the same treatment duration as required to reach drug uptake equilibrium. Increasing the treatment duration from 0.5 to 2 h was associated with an increase in drug uptake into stratum corneum from 0.03 to 0.06 $\mu g/cm^2$ and an decrease in the chromameter a scale from 7.3 to 6.3. Increasing the treatment duration further to 6 and 16 h, however, did not change the drug content in the treated skin but was associated with further decreases in chromameter a scale assessment of skin color. The mechanism responsible for this equilibrium delay between pharmacokinetics and pharmacodynamics is often cited as an active metabolite or the dissociation between the tissue target site of drug activity and the tissue compartment measured for drug concentration [16]. Whereas no active metabolites of betamethasone dipropionate were detected with the HPLC method in the current study, it is believed that the site of corticosteroid-blanching activity in the skin lies in the papillary dermis [17]. This skin layer was not sampled or analyzed for drug content in the present study and thus could provide a reasonable explanation for the delayed equilibrium relationship. Another possible mechanism for this relationship may be a circadian activity of the drug in skin producing the blanching response. Indeed, plotting the pharmacodynamic response over 24 h following application of a single dose of DLC cream at 1600 h reveals a maximal skin response occurring 8 h after drug application for all treatment durations (Fig 2). Whereas increasing the treatment durations increased the extent of the mean maximal response, those increases were not dose proportional or significantly different ($p > 0.05$). These data suggest that even very short durations of treatment with 0.05% DLC may produce the maximal pharmacodynamic response and that this maximal response occurs consistently at a particular time, 8 h after drug application, regardless of treatment duration.

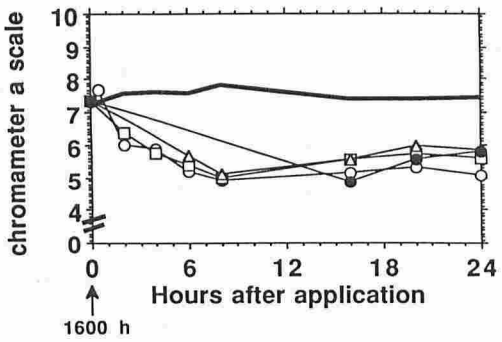

Figure 2. Profile of chromameter a scale skin color values over time. Changes in skin color at treated human ventral forearm skin sites were measured with the chromameter a scale at various times following a single unoccluded dose of 0.05% DLC applied for 0.5, 2, 6, and 16 h. *Bold line without symbols,* untreated control site; *open circle,* 0.5 h; *open square,* 2 h; *open triangle,* 6 h; *closed circle,* 16 h. Mean ± SEM, n = 6 subjects.

Reproducibility of the Chromameter-Assessed Blanching Response The time of the maximal decrease in skin color following a single topical dose of 0.05% DLC is reproducible (Fig 3). Six independent experiments over a 2-year period using many of the

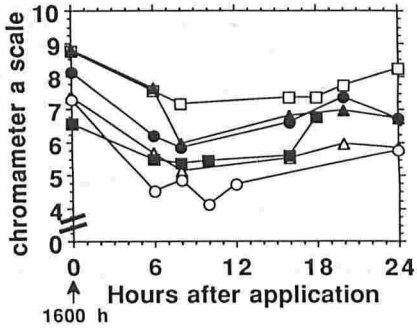

Figure 3. Reproducibility of the changes in skin color following a single 6-h unoccluded dose of 0.05% DLC. Skin color was measured at various time points over 24 h after drug treatment using the chromameter a scale. Mean ± SEM, n = 6 subjects. *Open circle,* drug-treated site in February 1990 study; *open square,* drug-treated site in August 1990 study; *open triangle,* drug-treated site in February 1992 study; *closed circle,* drug-treated site in an early March 1992 study; *closed square,* drug-treated site in a mid-March 1992 study; *closed triangle,* drug-treated site in a late March 1992 study.

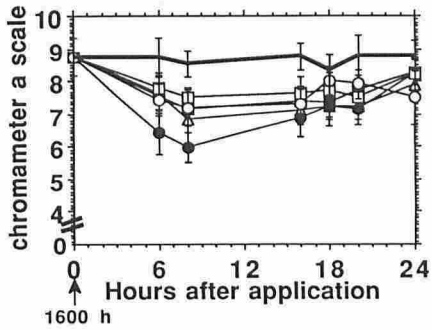

Figure 4. Profile of chromameter a scale skin color values on the ventral forearm over time following treatment with five different formulations of 0.05% betamethasone dipropionate. All formulations were applied for 6 h unoccluded, then removed and evaluated with a chromameter for changes in skin color at various times over the following 24 h. *Bold line without symbols,* untreated control site; *solid circle,* 0.05% DLO; *solid square,* 0.05% DLC; *open circle,* 0.05% DSO; *open square,* 0.05% DSC, *open triangle,* 0.05% DSL. Mean ± SEM, n = 6 subjects.

same subjects treated with a single 6-h unoccluded dermatologic dose of 0.05% DLC produced similar profiles of the skin response, independent of the time of year. It is interesting to note that baseline skin color is different throughout the year, likely due to sun exposure. Indeed, the same subjects have higher baseline chromameter a scale values on their ventral forearms during summer months than during winter months (data not shown). Nonetheless, the difference in the maximal response to drug treatment from baseline and the time of that maximal response (8 h after drug application) is similar, independent of the time of year.

Influence of Vehicle on the Skin-blanching Response For elucidation of whether the time of maximal decrease in skin color to 0.05% DLC is distinct to this cream formulation or generally applicable to this drug, various vehicle formulations of 0.05% betamethasone dipropionate were evaluated. Ten subjects were simultaneously treated with a single 6-h unoccluded dermatologic dose of two ointments (DLO and DSC), two creams (DLC and DSC), and one lotion (DSL). These vehicle formulations represent Stoughton [4] potency classes of I, II, III, and V (DLO, DLC, and DSO, DSC, and DSL, respectively). The influence of vehicle in the 0.05% betamethasone dipropionate formulation on the resulting maximal decrease in skin color and time of maximal decrease in skin color were objectively measured with the chromameter a scale as shown in Fig 4. Extent of the maximal decrease in skin color was vehicle dependent, in which the augmented ointment (DLO) decreased the chromameter a scale to a greater extent ($p < 0.05$) than the lotion (DSL), but both produced significantly greater ($p < 0.05$, ANOVA) maximal chromameter a scale decreases than either cream formulation (DLC, DSC) or the ointment (DSO) at 8 h. The three latter formulations, DLC, DSC, and DSO, were not significantly different ($p > 0.05$, ANOVA) from one another. None of the drug-induced alterations in skin color returned to untreated control values over the 24 h of measurement. The differential mean drug activity in the treated skin (chromameter a scale values) reflected the differential mean drug uptake into the rate-limiting barrier among these formulations [12], DLO ≥ DSL ≫ DLC = DSO = DSC; $p < 0.05$ ANOVA), thus supporting the hypothesis that increasing drug uptake into skin is associated with increased pharmacodynamic activity at the target site. The rank order of drug activity measured in the present study does not agree with that published by Stoughton [4], in which DLO > DLC = DSO > DSC > DSL using a one-time point determination only of the blanching response. Both methods apply a dermatologic dose of the drug formulation on the ventral forearm at 1600 h. The present method uses a 6-h duration of drug treatment and measures the resulting skin response periodically over time, documenting the maximal response occurring at 8 h post application. The modified McKenzie-Stoughton assay uses a 16-h drug treatment and measures the resulting response 18 h after drug application. Both methods measured the blanching response 2 h after drug removal. Disparity in the potency rank order of the various formulations between the two methods may reflect the different durations of treatment (6 h in the present study and 16 h in the Stoughton study), changes in the vehicle formulation by the manufacturer, or the time point after drug application chosen to measure the skin response to drug treatment.

Whereas different vehicle formulations of 0.05% betamethasone dipropionate produced different maximal extents of the pharmacodynamic response, the time of the maximal response occurred at the same time of day, approximately midnight, independent of the vehicle. These data suggest that optimal drug activity in the skin reflects both drug uptake into the skin influenced by the vehicle formulation and the inherent pharmacologic activity of the corticosteroid, as well as a time-dependent component.

Circadian Activity of Topical Corticosteroid Whether the time-of-day–dependent maximal activity of 0.05% betamethasone dipropionate is diffusion dependent or circadian dependent was further investigated in an *in vivo* experiment in which various durations (0.25, 0.5, 1, 2, and 6 h) of the same unoccluded dermatologic dose (2.6 μl/cm^2 skin) of 0.05% DLC were applied to one ventral forearm at 0900 h and the other forearm at 1600 h in 10 subjects. With this experimental design, if the time of the maximal decrease in skin color was merely the result of time required for drug to diffuse from the stratum corneum to the upper dermis, where the vascular response likely occurs [17], the maximal decrease in chromameter a scale values should occur at the same time after drug application with both the morning and afternoon applications. In contrast, if the skin response to the drug is the result of a chronobiologic-dependent process, the maximal response would occur at different times after drug application in the 0900-h and 1600-h groups, but at the same time of day, approximately midnight.

Comparison of the chromameter a scale profiles to various treatment durations of topical 0.05% DLC applied at 0900 h and 1600 h in all human subjects are shown in Fig 5*a,b,* respectively. Critical to the assessment of drug-induced changes in skin color are the endogenous changes in skin color at an untreated skin site (Fig 5, *bold solid line, no symbols*) over the same time period. Whereas skin color on the left versus right forearms at the same time point were not significantly different ($p > 0.05$), skin color at the untreated sites did vary over the 24-h period ($p < 0.05$, ANOVA). Untreated skin demonstrated maximal skin color (higher a scale values) with the chromameter a scale at noon and midnight in both left and right ventral forearms. Maximal decreases in chromameter a scale skin color occurred ~13 h after the 0900-h application with all treatment durations (0.25, 0.5, 1, 2, and 6 h) of 0.05% DLC. Maximal decreases in skin color occurred ~8 h after the 1600-h application with all treatment durations. Thus, the maximal decrease in skin color for the 0900-h application time occurred many hours longer after drug application than the 1600 h application time for all treatment durations. Assessment of skin color as actual time of day reveals that maximal decreases in the chromameter a scale measured skin color occurred between 2100 and 2300 h on day 1 for both the 0900-h and 1600-h applications. Therefore, these data demonstrate that topical 0.05% betamethasone dipropionate has maximal activity in the skin at a particular time of the day, approximately midnight, independent of the time of drug application.

Return of the decreased chromameter a scale values at drug-treated sites to those of untreated skin sites occurred within 24 h with all treatment durations in the 0900-h application group, but with the 0.25, 0.5, and 1 h durations only in the 1600-h application group. Longer treatment durations of 2 and 6 h applied at 1600 h produced significantly greater decreases in skin color ($p < 0.05$) that required longer periods of time to return to baseline than the

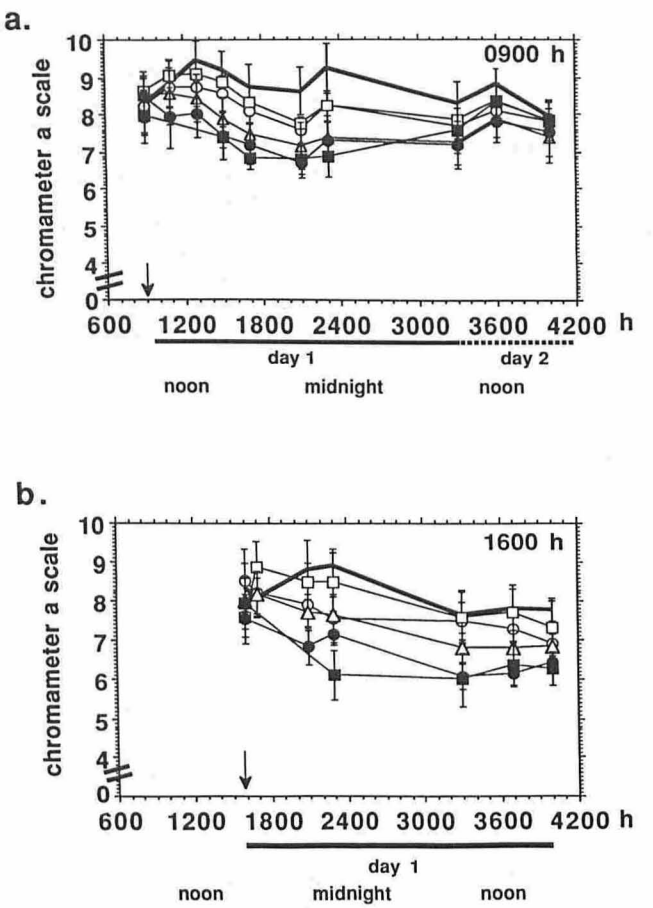

Figure 5. *a)* Profile of chromameter a scale skin color values following 0.05% betamethasone dipropionate cream applied at 0900 h. A single topical dose of 0.05% DLC was applied unoccluded at 0900 h to five different sites on the ventral forearm of each subject and removed after 0.25, 0.5, 1, 2, or 6 h. Changes in skin color were measured objectively with the chromameter a scale over 31 h after drug application. Data are plotted against actual time of day. *Bold solid line,* untreated control; *open circle,* 0.25-h treatment duration; *open square,* 0.5-h treatment duration; *open triangle,* 1-h treatment duration; *closed circle,* 2-h treatment duration; *closed square,* 6-h treatment duration. Mean ± SEM, n = 6 subjects. *Arrow,* time of drug application. *b)* Profile of chromameter a scale skin color values following application of 0.05% betamethasone dipropionate cream at 1600 h. A single topical dose of 0.05% DLC was applied unoccluded to five different sites on the opposite ventral forearm used in (*a*). Drug treatments were removed 0.25, 0.5, 1, 2, and 6 h later. Changes in skin color at those sites over the following 24 h were measured with the chromameter a scale. *Bold solid line,* untreated control; *open circle,* 0.25-h treatment duration; *open square,* 0.5-h treatment duration; *open triangle,* 1-h treatment duration; *closed circle,* 2-h treatment duration; *closed square,* 6-h treatment duration. Mean ± SEM, n = 6 subjects. *Arrow,* time of drug application.

0900-h application. These data suggest that application of the longer treatment durations of 0.05% DLC in the late afternoon produce greater activity for longer periods of time than application in the morning.

Analysis of the drug-induced changes in skin color over time can be assessed by calculating the area under the chromameter a scale time curve from 0 to 24 h (AUC_{0-24h}) using the trapezoidal rule [15]. Data demonstrate that 0900-h and 1600-h untreated control sites were not different ($p > 0.05$). In addition, all durations of treatment applied at 0900 h and 1600 h produced significantly greater AUC_{0-24h} values ($p < 0.05$) than the respective untreated control sites (Table I). Application of 0.05% DLC at 1600 h for 2

Table I. Influence of Time of Drug Application on Drug Activity in Skin Over 24 h Using Six Treatment Durations[a]

Time of Drug Application	Area Under the a scale Time Curve ($AUC_{0-24\,h}$) or Various Treatment Durations					
	0 h	0.25 h	0.5 h	1 h	2 h	6 h
0900 h	212.8 (12.4)	194.6 (7.7)	200.5 (5.5)	182.5[b] (11.8)	177.5[b] (11.3)	140.0[b] (9.2)
1600 h	197.0 (11.3)	180.7 (12.3)	183.4 (10.8)	174.0 (7.5)	137.0[b,c] (6.3)	103.1[b,c] (13.0)

[a] Mean (SEM), n = 6 subjects.
[b] $p < 0.05$ from 0 h, with ANOVA.
[c] $p < 0.05$, 0900 h versus 1600 h with Wilcoxon signed-rank test.

and 6 h, however, produced significantly greater pharmacodynamic activity (lower $AUC_{a\,scale\,0-24h}$) than the same treatment durations applied at 0900 h ($p < 0.05$). Thus, optimal drug activity in the skin is dependent not only on drug uptake and vehicle formulation but also on the time of day it is applied.

Circadian activities of other drugs have been previously demonstrated, notably the antineoplastic drugs cisplatin and doxorubicin [18,19]. Administration of these oral antineoplastic drugs in accordance with a circadian schedule maximizes their drug activity and reduces their toxic side effects. The circadian activity of betamethasone dipropionate in the present study differs from the oral antineoplastics in that it is administered topically and has the additional complexity of appearing to compete with endogenous circulating cortisol for activity in the skin. Indeed, the time of maximal betamethasone dipropionate activity in skin, around midnight, coincides with the well-known minimal circulating blood concentrations of endogenous cortisol that occur between 2000 and 0400 h [20]. Given that topical betamethasone dipropionate produces its maximal inherent pharmacodynamic response in the late evening through early morning hours, independent of vehicle formulation, treatment duration, or time of application, maximal delivery of drug to skin at times of low circulating cortisol (between 1600 and 2400 h) may provide greater therapeutic benefit over prolonged periods of time. Maximizing a drug's inherent pharmacologic activity by understanding the circadian activity of the endogenous substrates that compete with it for specific receptor binding in the target tissue may also reduce the frequency of dosing required to treat disease in that tissue. Reducing the total amount of drug applied over a 24-h period may also have the benefit of reducing the toxic side effects to the skin associated with this class of drugs.

Understanding the circadian activity of topical corticosteroids is also critical to bioequivalence assessment.† Identification of the time of maximal drug activity after topical corticosteroid application and the length of that activity over a 24- to 48-h time period may have a significant impact on the determination of bioequivalence between two drug formulations. The circadian activity and time of maximal skin response to a particular topical corticosteroid will dictate the experimental design for bioequivalence testing. Objective measure of the skin-blanching response at multiple time points is therefore necessary to identify the time, extent, and duration of the drug-induced activity.

In summary, these data collectively demonstrate the circadian effects of a topical corticosteroid in human skin *in vivo.* Further elucidation of these circadian pharmacodynamic responses with other topical corticosteroids may provide a rational basis for the experimental design of bioequivalence assessment of various formulations,† as well as re-evaluation of the appropriate therapeutic

† *FDA Guidelines for Bioequivalence Testing of Topical Corticosteroids,* July 1992. Office of Generic Drugs, 7500 Standish Place, Rockville, MD 20855.

regimen to maximize drug activity and reduce toxic side effects of this drug class in dermatologic medicine.

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