Feasibility of Measuring the Bioavailability of Topical Betamethasone Dipropionate in Commercial Formulations Using Drug Content in Skin and a Skin Blanching Bioassay

Lynn K. Pershing,[1,3] Barbara S. Silver,[1] Gerald G. Krueger,[1] Vinod P. Shah,[2] and Jerome P. Skelley[2]

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An *in vivo* technique has been developed which simultaneously compares a skin blanching bioassay with drug content in human stratum corneum following topical application of four 0.05% betamethasone dipropionate formulations. Bioavailability of drug from commercial cream and ointment formulations was assessed by quantification of drug content in tape-stripped stratum corneum and skin blanching in the treated skin site under occluded conditions. Tape-stripping removed stratum corneum to a varying degree between individuals but was consistent (35%) within an individual with all formulations, day to day. A correlation (r = 0.9935) between the amount of drug in the treated stratum corneum normalized for surface area and the corresponding skin blanching score was observed with four 0.05% betamethasone dipropionate formulations. Increasing the amount of drug in the tape-stripped stratum corneum correlated with an increased skin blanching score. Ointment formulations delivered more drug to the skin and produced greater blanching scores than the cream formulations. Topical corticosteroid content in the treated skin site can therefore be quantified and correlates well with the resulting pharmacodynamic activity.

KEY WORDS: bioavailability; skin blanching; vasoconstriction; topical corticosteroids; betamethasone dipropionate; tape-stripping.

INTRODUCTION

Considerable literature exists comparing the potency and bioavailability of topically applied corticosteroids *in vivo* using clinical efficacy (1–3) and vasoconstriction, also known as skin blanching (4,5). These studies have proven useful for ranking corticosteroid potency (6–8) and for distinguishing between commercial formulations (9). The quantification of vasoconstriction or skin blanching, however, is subjective. Further, the methods of drug application, the time point of skin blanching assessment, and the criteria used to rank the blanching response vary among investigators (4,10–12). An analytical method, which objectively quantitates bioavailability as a function of the amount of drug within the treated stratum corneum site, would provide greater insight into differences among formulations observed with the blanching assay and could also be used as a basis to standardize the bioavailability testing of this drug class. Such a chemical assay of this type, however, needs to be validated with an existing bioassay.

The study presented herein explores the feasibility of simultaneously correlating the amount of topically applied corticosteroid in stratum corneum and its pharmacological activity *in vivo*, i.e., skin blanching. This approach is based upon the assumption that effective cutaneous therapy requires partitioning of the topically applied drug from its vehicle formulation into the stratum corneum. The amount of drug partitioning into the stratum corneum can be assessed by tape-stripping the same and chemically quantitating the amount of drug in the tape strips. This approach has been successfully used to predict total drug absorption across skin *in vivo* (13).

The strength of the correlation between drug content in stratum corneum and *in vivo* pharmacological activity, i.e., skin blanching, will demonstrate whether an interrelationship between these two parameters exists and will provide a greater basis of understanding of the discrepancies of clinical efficacy among various chemically equivalent commercial products.

MATERIALS AND METHODS

Human Skin Model

Five Caucasian human volunteers, male and female, ages 27–48 years of age, naive to topical drug therapy were used for these studies. The volunteers were not prescreened for sun exposure or for use of sunscreens; however, their skin pigment was unchanged over the course of the study period. Two commercial cream and ointment formulations of 0.05% betamethasone dipropionate (Table I) were analyzed for their ability to partition into human stratum corneum *in vivo* and induce a skin blanching response. All 0.05% betamethasone dipropionate formulations were applied to the same human subject forearm at the same time on right and left forearms.

Protocol for Application of Formulation

The same dose, 180 mg of each formulation, was placed in a 1.2-cm-diameter Hilltop chamber (Hilltop Research Inc., Cincinnati, OH) and affixed via the supplied adhesive tape to a 1.13-cm^2 surface area on the volar aspect of the forearms. This dose, approximately 159 mg formulation/cm^2, is 10 times greater than the average dose applied clinically. Larger doses with occlusion were utilized in the present study to ensure sufficient analytical sensitivity of the drug uptake into the stratum corneum. A maximum of three chambers, equally spaced 2 cm apart, was applied to each forearm for each study, being careful that the chambers were a minimum of 6 cm above the wrist and a minimum of 6 cm below the antecubital fossa (Fig. 1) to minimize the disturbance of the drug treatment throughout normal daily activity. The order and placement of creams or ointments applied to the forearm were randomized. All experimental skin sites were maintained under occlusion for 24 hr. Application of

[1] Division of Dermatology, University of Utah, Salt Lake City, Utah 84132.
[2] Center for Drug Evaluations and Research, Food and Drug Administration, Rockville, Maryland 20857.
[3] To whom correspondence should be addressed.

Table I. Topical Corticosteroid Formulations Tested

Formulation	Name	Manufacturer
0.05% betamethasone dipropionate cream	Diprolene Advance Formula	Schering
	Maxivate	Westwood
0.05% betamethasone dipropionate ointment	Diprolene Actibase	Schering
	Maxivate	Westwood

the same formulation to the right or left forearm produced stratum corneum concentrations and resulting skin blanching scores that varied by 35 and 8%, respectively (data not shown). The variability in the drug content in the stratum corneum and the skin blanching score within the same person was equal or less than the variability between all persons. Thus, no preference was given to the random application of the various formulations to either arm.

The occluded state was selected because it (i) protected the drug-treated skin site from the environment, thus minimizing the possibility of drug removal, and (ii) maximized drug partitioning into the skin, thus maximizing the analytical detection and subsequent quantification of drug in the stratum corneum.

Tape-Stripping Protocol

The Hilltop chamber was removed at the end of the dosing interval (24 hr) and residual formulation on the skin surface was gently removed with a Teflon spatula. The drug-treated site was then gently wiped with three independent dry cotton applicators and allowed to air-dry for 1–3 min. Ten individual 0.6-cm-diameter disks of tape (Transpore; 3M, St. Paul, MN) were utilized to tape-strip the center of the 1.2-cm-diameter drug-treated skin site (Fig. 1). Each of these disks was weighed on an Ultramicro balance (Sartorius MP8-1; Brinkmann Instruments, Westbury, NY) before and after skin tape-stripping to quantitate the weight of stratum corneum removed (sensitivity of 0.1 μg). Two sequential tape-strip disks were combined for extraction and submitted to high-pressure liquid chromatography (HPLC) for quantitation of drug content, thereby providing five stratum corneum samples per treated skin site.

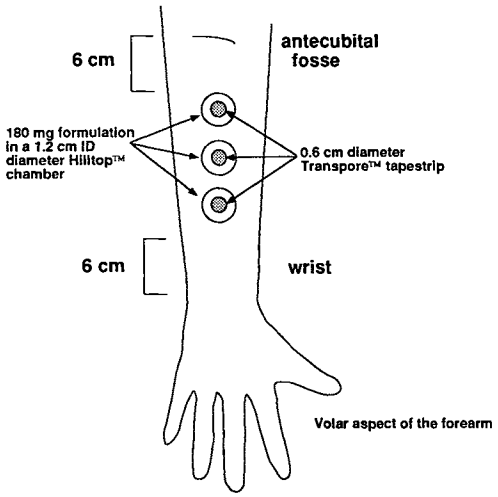

Fig. 1. The human volar forearm skin test site. The drug treatment consists of dispensing the test formulation into a Hilltop chamber and placing the chamber within the defined area, 6 cm below the antecubital fossa and 6 cm above the wrist. The drug-treated site is subsequently tape-stripped after 24 hr and skin blanching is monitored over time (see Materials and Methods).

Preliminary experiments showed that tape-stripping a 6-mm-diameter site in the center of the 1.2-cm-diameter drug-treated area did not alter the skin blanching produced by topically applied corticosteroids as a function of time.

Skin Blanching Scoring

Skin blanching was monitored in the remaining drug-treated skin site after tape-stripping. Skin blanching was scored using a 0–4 scale (see below) by a single investigator at 1, 24, and 48 hr after drug removal.

0 = Normal skin
1 = Slight, diffuse blanching with indistinct outline
2 = More intense blanching with half of the drug-treated site perimeter outlined
3 = Marked blanching with a distinct outline of the drug-treated skin site
4 = Extreme blanching with a distinct outline of the drug-treated skin site

Extensive skin blanching produced by topical corticosteroids in human subjects has been associated with increased clinical efficacy (1). The ability of a topical corticosteroid to induce extensive skin blanching over prolonged periods of time may therefore be used as a method to differentiate further the bioavailability among commercial formulations of the same drug (7,8,14,15). This approach has been reduced to practicality by combining the effects of time and skin blanching into a composite skin blanching score. The skin blanching score utilized in the present correlation study assumes that the greater the amount of drug remaining in the skin over time, the more prolonged a pharmacological effect. The skin blanching score was therefore calculated additively using the time interval of observation (hours) multiplied by the extent of skin blanching at that time interval. For example, an extent of skin blanching at 1, 24, and 48 hr of 4, 3, and 1, respectively, produces a score of 124: $[(1) \times (4) + (24) \times (3) + (48) \times (1)] = 124$.

High-Pressure Liquid Chromatography (HPLC) Assay

Betamethasone dipropionate in the various samples was identified chromatographically at a retention time of 6.45 min by HPLC (Beckman binary gradient Model 334) on a C-18 RP column (4.6 × 25 mm; Altex, Palo Alto, CA) at 25°C, using a 1.2 ml/min delivery of degassed acetonitrile: distilled water (65:35 v/v) mobile phase and a 20-μl sample loop (Fig. 2). Drug in tape-stripped stratum corneum was detected from an absorbance spectra of betamethasone dipropionate at 254-nm UV fixed wavelength detection (Beckman model 164). Drug concentrations in the tape-stripped samples were determined from dipropionate stan-

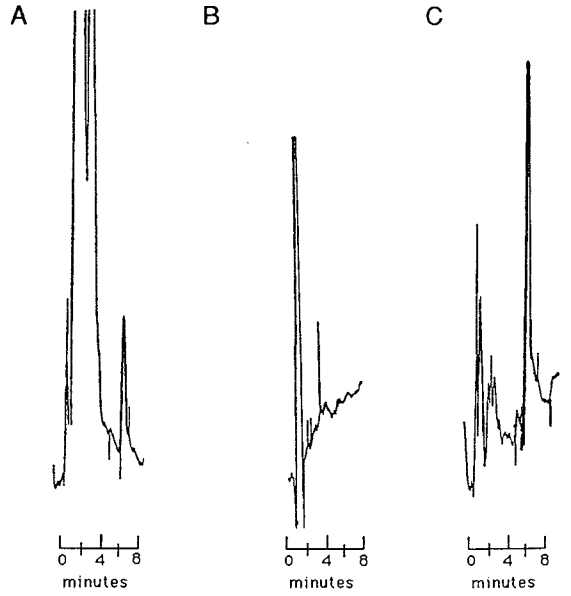

Fig. 2. The HPLC chromatogram of betamethasone dipropionate in pure standards and skin. (A) Betamethasone dipropionate, 0.5 μg/ml; RT = 6.45 min (chart speed, 0.10 cm/min). (B) Acetonitrile extract of skin + tape. (C) Acetonitrile extract of skin + tape + betamethasone dipropionate.

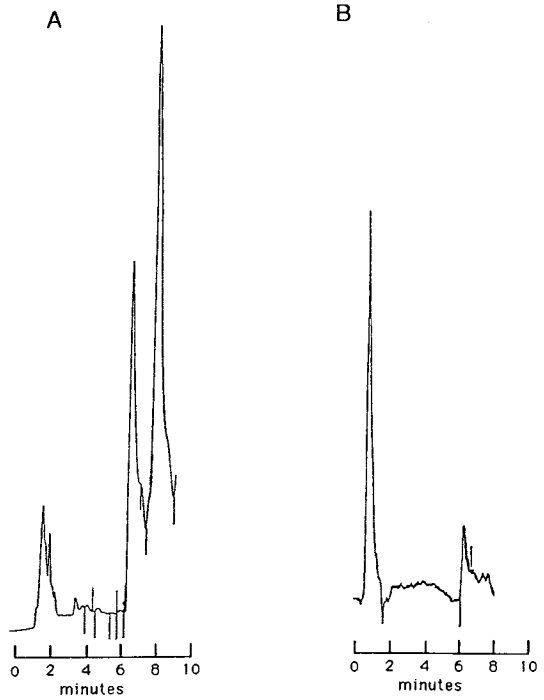

Fig. 3. HPLC chromatogram of betamethasone dipropionate and unknown. Retention times of betamethasone dipropionate and unknown are 7.93 and 6.56 min, respectively. Flow rate, 1.2 ml/min; chart speed, 0.25 cm/min.

dard curves (0–1 mg/ml) generated with the pure compound, generously supplied by Schering Corporation (Kenilworth, NJ). The limit of sensitivity of betamethasone dipropionate was 0.05 μg/ml or 1 ng on column. The standard curves were linear for betamethasone dipropionate ($r = 0.9993 \pm 0.0015$; mean ± SD for $n = 8$) with an interrun precision (coefficient of variation) of 0.15%.

Two sequential tape strips were combined in a clean, labeled 1.5-ml polypropylene microcentrifuge tubes and 200 μl of acetonitrile (HPLC grade; Burdick and Jackson) was added for extraction of drug. The tubes were capped, vortexed for 1 min at high speed, and centrifuged at 5000 rpm for 5 min (Biofuge A; Heraeus, West Germany). The supernatant was transferred into clean labeled microcentrifuge tubes and 2.5 times the sample loop volume was injected onto the HPLC, to ensure a full loop sample. A single extraction of the tape strips containing drug and stratum corneum was sufficient to recover routinely >90% of the drug.

Chromatographic analysis of tape and tape + skin extractions did not reveal any interfering substances with betamethasone dipropionate (Fig. 2). An unknown peak was identified in the tape-stripped betamethasone dipropionate-treated stratum corneum samples that chromatographed 1–1.5 min before betamethasone dipropionate (Fig. 3). Efforts to identify the compound revealed that it was not the betamethasone moiety nor the 17- or 21-monopropionate, a component of the vehicle, nor an interference from the skin itself. The component was not present in pure standards stored at 4°C in our facility until after 3 months of storage in the refrigerator. It was present in the extractions of all commercial 0.05% betamethasone dipropionate formulations, but to a greater extent in the ointment formulations. Further, the material maximally absorbed UV light at 269 nm, ~33 nm higher than the peak absorbance of the other betamethasone derivatives. The unknown peak could be a degradation product of betamethasone dipropionate, which may be generated during the extraction process. Regardless of its chemical description, ~1.5 μg of the unknown produced a blanching response of 3–4 after 24 hr of occlusion in a Hilltop chamber. For this reason, the drug content in the betamethasone dipropionate-treated skin presented herein reflects the sum of betamethasone dipropionate and an unknown material.

Calculations

Drug concentrations (μg/ml) of betamethasone dipropionate and unknown in the tape strips were quantitated against pure standard curves by the area-under-the-curve method (AUC). Drug content (μg) in the tape-stripped skin was normalized for individuals by dividing the total drug content in each drug test site by the surface area of the tape strips. The drug content in individual skin sites was also normalized with the total amount of stratum corneum removed in 10 tape-strippings. These normalizing techniques ensure that the variability in drug concentration between different skin sites on the same individual or between individuals is a result of actual differences in drug partitioning into the skin, and not merely the result of collecting greater or lesser amounts of tissue. A comparison between the results normalized with surface area or amount of stratum corneum weight removed with tape-stripping was not statistically different. Statistical significance ($P < 0.05$) between the skin concentrations of drug and/or between skin blanching scores generated by the various formulations tested was determined using a two-tailed Student's t test.

RESULTS

Stratum Corneum Weight Removed with Tape Strips: *In Vivo*

Cumulative weights of stratum corneum removed with the tape-stripping technique following 24 hr of occlusion with a 0.05% betamethasone dipropionate cream (Westwood) and ointment formulation (Schering) are presented in Figs. 4A and B, respectively. The profile of stratum corneum removed from each of the three illustrative subjects is linear with both drug treatments, demonstrating a constant amount of stratum corneum removed with each tape strip. The amount of stratum corneum removed with each tape strip may differ, however, as much as threefold between these individuals, as witnessed by the different slopes and total amount of skin removed in the cumulative weight profiles.

Although differences in the amount of stratum corneum removed with each tape strip exists between individuals, the profile of the amount of stratum corneum removed from a particular individual is relatively constant from day to day, regardless of the cream or ointment formulation tested. This is illustrated by the similarity in the profile and the total amount of stratum corneum weight removed from a particular subject (No. 1 or 2 or 3) following 24 hr of exposure to 0.05% betamethasone dipropionate cream (Westwood) (Fig. 4A) and ointment (Schering) (Fig. 4B). The total weight of stratum corneum removed with 10 tape-strippings can also vary as much as threefold between these subjects. The total stratum corneum weight removed from subject 1 is consistently greater than that from subject 2, which is consistently greater than that from subject 3. The mean total stratum corneum weight removed with 10 tape strips of a 0.6-cm diameter from these individuals following treatment with the cream and ointment formulations, 283 ± 82 and 327 ± 111 μg (mean + SD; n = 3), respectively, was not statistically different (Fig. 5). The mean accumulative profile of stratum corneum removed is linear over 10 tape strips (r = 0.9960) and confirms that, within the population of individuals studied, a constant amount of skin weight is removed, 323 μg. Since the tape-stripped surface area is the same for all sites, and the profile or total amount of skin weight removed in that given surface area is very reproducible per individual, and the same individual is used for all formulations evaluated, bioavailability differences among formulations can be easily determined by normalizing the drug content in the tape-stripped stratum corneum samples with the surface area of the tape-stripped site only. This method of normalization has the added advantages of being investigator and equipment independent, thus enabling evaluation of data from multiple centers.

Correlation Between Skin Weight and Removal of Stratum Corneum with Tape-Stripping

Prior to conducting the quantitation studies of drug content in tape-stripped stratum corneum, the thoroughness of stratum corneum removal by tape-stripping with 0.6-cm-diameter Transpore tape 10 times was analyzed by histologic examination of a biopsy collected from a fully hydrated human abdominoplasty skin specimen that had been tape-stripped in this manner. This analysis compared the stratum corneum weight removed *in vitro* with the layers of stratum corneum remaining as a function of tape-stripping. The tape-stripping technique removed stratum corneum from the abdominoplasty specimen *in vitro* in a cumulative profile that fit a second-order polynomial equation (Fig. 6). The first five tape strips contained the greatest amount of stratum corneum (200 μg), with a maximum total weight of 300 μg in 10 tape-strippings. These data contrast with the *in vivo* counterpart, where each tape strip removed the same amount of

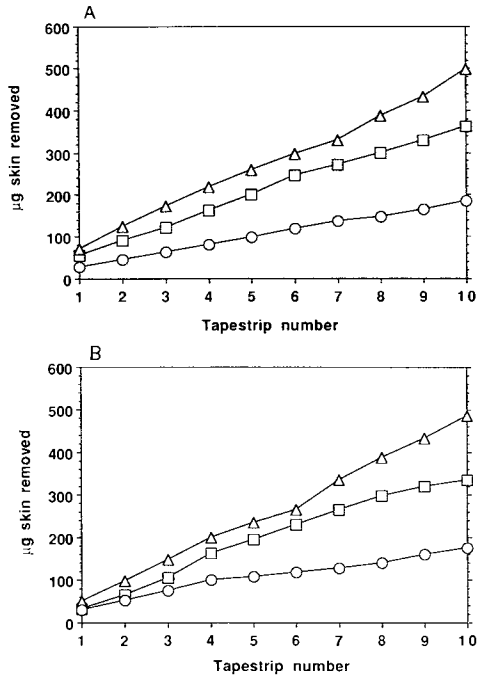

Fig. 4. (A) Cumulative skin weight removed with tape-stripping human volar forearm skin from three subjects following a 24-hr exposure to 0.05% betamethasone dipropionate cream (Westwood). Open triangle, subject 1; open square, subject 2; open circle, subject 3. (B) Cumulative skin weight removed with tape-stripping human volar forearm skin from three subjects following a 24-hr exposure to 0.05% betamethasone dipropionate ointment (Schering). Open triangle, subject 1; open square, subject 2; open circle, subject 3.

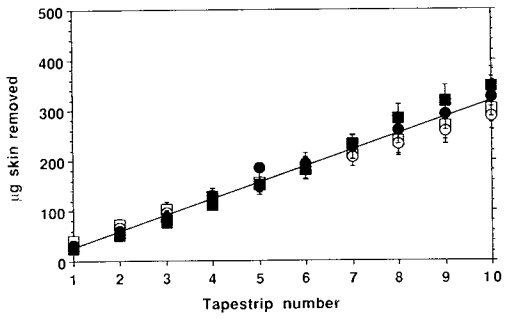

Fig. 5. Cumulative skin weight removed from tape-stripped sites treated with 0.05% betamethasone dipropionate cream and ointment formulations. Data represent the mean and SEM for three subjects. Open circle, 0.05% betamethasone dipropionate cream (Schering); open square, 0.05% betamethasone dipropionate cream (Westwood); filled circle, 0.05% betamethasone dipropionate ointment (Schering); filled square, 0.05% betamethasone dipropionate ointment (Westwood).

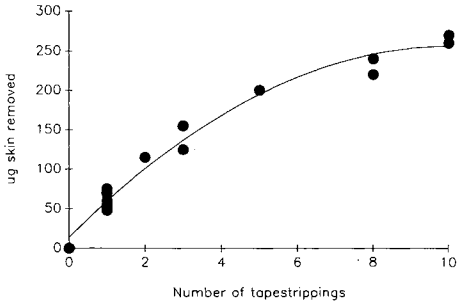

Fig. 6. Cumulative skin weight removed from female abdominal skin with 1–10 tape strips *in vitro*.

stratum corneum (see Figs. 4A and B). Nevertheless, the total amounts (μg) of stratum corneum removed *in vitro* and *in vivo* with the tape-stripping technique were similar.

Prior to tape-stripping, histology of a fully hydrated human abdominoplasty skin *in vitro* displayed an intact stratum corneum of ~10 layers. Removal of 125–200 μg of stratum corneum, ignoring the water weight in the hydrated abdominoplasty skin, corresponded with the removal of approximately four layers of stratum corneum histologically (Fig. 7). Removal of 250 μg of stratum corneum corresponded with removal of approximately eight layers of the stratum corneum. Ten tape-strippings of human forearm skin *in vivo* removed ~275–300 μg of stratum corneum and resulted in glistening at the tape-stripped skin site. Complete removal of the stratum corneum *in vivo* is commonly recognized by a glistening appearance of the tape-stripped site. These comparative analyses confirm that 10 tape strips of 0.6-cm-diameter Transpore tape is sufficient to remove the majority of human stratum corneum *in vivo* and *in vitro*.

Skin Blanching Profiles

The cream and ointment formulations containing 0.05% betamethasone dipropionate were applied under occlusion for 24 hr. At that time, excess drug was removed and skin blanching was measured at three time points: 1, 24, and 48 hr post drug removal. The extent of skin blanching 1 hr post drug removal did not reveal significant differences between the various formulations of betamethasone dipropionate. However, both Schering and Westwood ointment formulations demonstrated a significant increase ($P < 0.05$) in the extent of skin blanching relative to the creams (Fig. 8) 24 and 48 hr post drug removal. These data demonstrate that measuring the extent of skin blanching over multiple time points

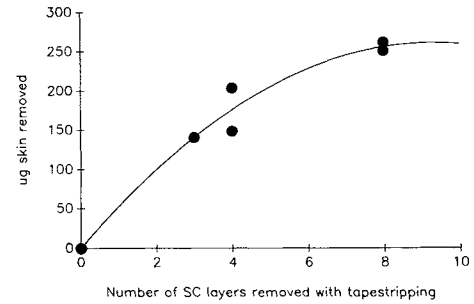

Fig. 7. Correlation of skin weight removed from female abdominal skin with the number of stratum corneum layers removed *in vitro*.

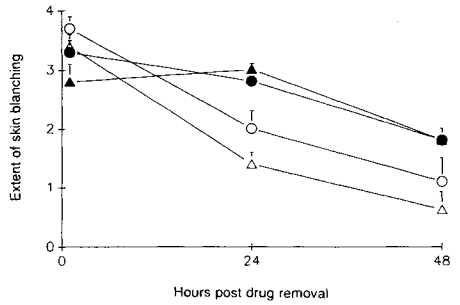

Fig. 8. Skin blanching profiles of 0.05% betamethasone dipropionate following 24 hr of occluded exposure. Data represent mean ± SE for five subjects. Open triangle, Westwood cream; open circle, Schering cream; filled triangle, Westwood ointment; filled circle, Schering ointment.

can further differentiate the bioavailability of various formulations of the same drug.

Correlation Between Amount of Drug in the Tape-Stripped Stratum Corneum and Skin Blanching

Skin blanching was quantitated in the present study as the extent of skin blanching as a function of time. The composite skin blanching scores (see methods) were plotted against the amount of drug within the tape-stripped stratum corneum (μg drug/cm^2 surface area) from five or more individuals who received 24 hr of occluded exposure to 0.05% betamethasone dipropionate cream and ointment formulations. A good correlation ($r = 0.9935$) was demonstrated (Fig. 9) between the amount of drug within the stratum corneum at the end of the 24-hr dosing interval and the resulting skin blanching score. Increasing the amount of betamethasone dipropionate in the stratum corneum was associated with a higher skin blanching score. Ointment formulations of 0.05% betamethasone dipropionate from both commercial suppliers (filled circle and filled square) produced greater concentrations in the stratum corneum and greater skin blanching scores than their respective cream formulations (open circle and open square). Both cream and ointment formulations of 0.05% betamethasone dipropionate

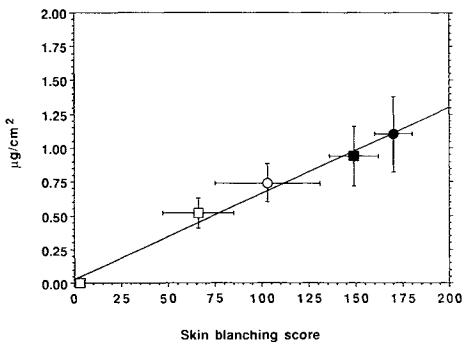

Fig. 9. Correlation between the amount of betamethasone dipropionate in skin corrected for surface area of tape-stripped area (μg/cm^2), and the skin blanching score ($r = 0.9935$). Data represent mean ± SE for five subjects. Open triangle, untreated control; open circle, Schering cream; filled circle, Schering ointment; open square, Westwood cream; filled square, Westwood ointment.

have greater amounts of drug in the tape-stripped stratum corneum and skin blanching scores than the untreated control site, which was occluded with the Hilltop chamber only.

DISCUSSION

The feasibility of assessing bioavailability of topical corticosteroids *in vivo* with an objective chemical quantitation of the treated stratum corneum was investigated with four topical 0.05% betamethasone dipropionate formulations. This approach requires a sensitive analytical method and an efficient, reproducible method for removing the stratum corneum *in vivo*. The tape-stripping method used in the current study removes greater than 90% of human stratum corneum *in vivo* and *in vitro* in a reproducible manner, without inflicting undue discomfort. The amount of stratum corneum removed is constant within the test human population (~323 μg) despite the vehicle formulation applied. Drug content in the tape-stripped skin can therefore be easily normalized with the surface area of the tape strip.

Validation of this quantitative approach for assessing topical drug bioavailability was investigated by correlating the mean quantity of drug in human stratum corneum *in vivo* with its mean pharmacological response, i.e., skin blanching, at the same skin site at three time points after drug removal, 1, 4, and 24 hr. Increasing the amount of betamethasone dipropionate in the human stratum corneum resulted in greater skin blanching scores, thus supporting the hypothesis that the amount of drug in the rate-limiting barrier will predict the pharmacodynamic activity of the topical corticosteroid.

The two ointment formulations of 0.05% betamethasone dipropionate in the present study delivered significantly more drug to the stratum corneum ($P < 0.05$) and produced greater skin blanching scores than the two cream formulations. These data agree with the general clinical experience that ointments are more potent than cream formulations of the same drug (6,9,18–20). The lack of statistical significance between the two cream formulations or the two ointment formulations in the present study could be secondary to the occlusive state of the treated skin site. Occlusion has been demonstrated to increase the skin blanching response in human subjects treated for short time periods (6 hr) with both cream and ointment formulations of betamethasone valerate and dipropionate, fluocinolone acetonide, triamcinolone acetonide, and fluocinonide (18,20). Poulsen and Rorsman (18) demonstrated that occlusion increased the skin blanching response to the greatest extent with low-potency corticosteroids.

Occlusion might also increase the drug concentration at the corticosteroid receptor, thus minimizing differences between formulations that might otherwise be observed in nonoccluded conditions. Indeed, Ostrenga *et al.* (20) noted a more clear differentiation in the skin blanching response between five cream and five ointment formulations of fluocinonide in the nonoccluded state compared with the occluded state. Clear differentiation among the cream and ointment formulations in the nonoccluded state was observed primarily, however, at early time points after drug removal in the formulations with a low fluocinonide solubility. The effect of occlusion on skin blanching is therefore dependent on the vehicle formulation, drug solubility in the vehicle formulation, and potency of the corticosteroid. Whether a more clear differentiation between the 0.05% betamethasone dipropionate vehicle formulations can be demonstrated under nonoccluded conditions with these methods is the subject of a current investigation. Nonetheless, the rank order of both the mean amount of drug in the tape-stripped stratum corneum and the mean skin blanching score agree with the rank order of these same formulations established by Cornell and Stoughton (1).

Variability between subjects in the amount of betamethasone dipropionate in the tape-stripped human stratum corneum from both creams and ointments is similar, ~50%. The inherent 30–50% variability in the present study is similar to that observed in percutaneous absorption of various compounds across different human skin sources (14). Variability in the skin blanching scores following topical application of cream (63%) and ointment (17%) formulations is similar to the 30–50% variability in other nonoccluded skin blanching studies with both ointment and cream formulations (9). The lower variability in skin blanching scores with the ointment formulations in the present study likely reflect maximal drug delivery and binding to the corticosteroid receptor under occlusion. These data emphasize that the pharmacological effect of topical corticosteroids is highly dependent on the concentration of the drug at the target site and the corticosteroid receptor, both of which are subject dependent. Given this potential for variability in both physicochemical parameters (partitioning) and pharmacological responses (skin blanching), it is remarkable that a correlation exists in the current study between the mean drug content within the drug-treated skin site and the mean skin blanching score. Comparison of the various formulations of 0.05% betamethasone dipropionate, however, reveals an excellent correlation ($r = 0.9935$) between these parameters. This correlation between drug content in the stratum corneum and skin blanching is in harmony with a recent report using two hydrocortisone cream formulations *in vivo* (17).

In summary, a method has been developed to quantify objectively drug bioavailability *in vivo* in tape-stripped human stratum corneum following topical application of corticosteroids. Drug content in the treated human stratum corneum (μg/cm^2) correlates well with its pharmacodynamic activity (skin blanching) at the same skin site. Differences observed in drug content in the stratum corneum and skin blanching agree well with the clinical efficacy of these products (1,6) and the rank order of these formulations previously established by Cornell and Stoughton (1). The objective chemical analysis of the treated stratum corneum for drug content is, therefore, a feasible method with which to assess bioavailability of topical betamethasone dipropionate. This approach will likely have utility in addressing topical corticosteroid bioavailability, including the influence of vehicle, drug concentration, and/or solubility in the vehicle, molecular variation of the corticosteroid structure, and occlusion.

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