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RESEARCH PAPER

Assessing Topical Bioavailability and Bioequivalence: A Comparison of the In vitro Permeation Test and the Vasoconstrictor Assay

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ABSTRACT

Purpose To compare the sensitivity of a pharmacokinetic assay, the *in vitro* permeation test (IVPT), with that of a pharmacodynamic assay, the human skin blanching or vasoconstrictor (VC) assay, in assessing the relative bioavailability of topical clobetasol propionate products.

Methods The percutaneous absorption of clobetasol propionate from five commercial products was measured *in vitro* using cryopreserved human skin. The pharmacodynamic potency of the same five products was also assessed *in vivo* using the VC assay, the surrogate method by which regulatory authorities in the United States establish the bioequivalence of generic topical glucocorticoid products.

Results IVPT found total clobetasol absorption varying ten-fold from highest to lowest product, whereas the VC assay found this same difference was less than two-fold. The coefficient of variation ranged from 78 to 126% in the VC assay, but only 30–43% for IVPT. Statistically, IVPT could separate the 5 products into three groups: 1) ointment, 2) cream and gel, 3) emollient cream and solution). Due to its greater variability as well as saturation of the pharmacodynamic response at higher flux levels, the VC assay found all products except the solution to be equipotent.

Conclusions IVPT was found to be substantially more sensitive and less variable than the VC assay for assessing clobetasol bioavailability.

KEY WORDS Clobetasol propionate · Bioavailability · Bioequivalence · *In vitro* permeation test · Vasoconstrictor assay

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INTRODUCTION

The long history of use of excised human skin as an accepted *in vitro* model for the study of percutaneous absorption has logically led to its consideration as a surrogate for clinical trials or human pharmacokinetic studies in determining the bioavailability (BA) and bioequivalence (BE) of topical drug products [1]. The US Food and Drug Administration (FDA) originally explored this possibility in 1986 but concluded that insufficient supportive data were available at the time to officially accept the model and that "more experience" with this approach for issues related to BA/BE was required [2].

Since that time use of the model has greatly expanded and led to the emergence of new data that present a consistent picture confirming the relevance and accuracy of *in vitro* percutaneous absorption data . In a survey of the literature to assess the correlation between *in vitro* and *in vivo* permeation data, Lehman *et al*. identified 92 data sets in which the absorption of 30 compounds had been assessed both *in vitro* and *in vivo* [3]. Most of the data came from studies lacking complete concordance between the *in vitro* and *in vivo* protocols and it was found that the *in vitro*/*in vivo* (IVIV) ratio for any single compound was highly variable, ranging from 0.18 to 19.7. Nevertheless, the average ratio for the group as a whole was relatively good at 1.6. Subsequently, a subset of the data comprising eleven compounds in which the two protocols were identical was identified. In this case the IVIV ratio for the group was found to approximate one (0.96) and, more importantly, for any single compound the ratio varied from only 0.58–1.28. The excluded data came from studies in which different anatomical sites were utilized and/or in which the test drugs were applied from different vehicles, both being well known factors that significantly affect the rate of absorption. Considering that IVIV correlation inevitably involves different groups of subjects, the high degree of correlation and relatively low variability noted in the subset is especially significant.

In addition to the above analysis that affirms the validity of the *in vitro* model as a surrogate for assessing systemic BA and, by inference, local BA; specific application of the model as a surrogate for demonstrating the BE of topical drugs has also been evaluated [4]. The relative BA of seven prospective generic drug products, in comparison to the corresponding reference listed drugs (RLD), was assessed during their preclinical development phase for the specific purpose of confirming "*in vitro* BE" prior to initiating more costly *in vivo* BE trials. Absorption of the active pharmaceutical ingredient (API) from both test and reference products (five glucocorticoids, two retinoids) was compared side-by-side in the *in vitro* permeation model. Six of the seven test products were found to be equivalent to their respective RLD, with test/reference ratios for total absorption ranging from 0.96–1.14. Following this, the test products were evaluated by clinical trial (retinoids), or by vasoconstrictor assay (glucocorticoids), where their BE was established according to current US regulatory standards. *In vitro* assessment of the seventh test product, the glucocorticoid mometasone furoate, found a test/reference ratio of only 0.63; yet, by vasoconstrictor assay, it too was found to be BE to the reference product (test/reference = 1.11) and subsequently approved. The apparent lack of agreement between the permeation and clinical results in this one instance was judged to be the result of the greater sensitivity of the permeation model relative to the VC assay, as had been seen before with the glucocorticoid, betamethasone valerate [5].

The vasoconstrictor or skin blanching assay is a pharmacodynamic assay dependent on the ability of glucocorticoids to cause constriction of the superficial blood vessels of the skin. First introduced by McKenzie and Stoughton [6], the method was modified and issued as a guidance by the FDA in 1995 to supplant the use of clinical trials to demonstrate the BE of topical glucocorticoids [7]. It is currently the only surrogate test accepted by the agency that is applicable to an entire class of topical drug products (with the exception of glucocorticoid shampoo products that are not solutions). Because of the aforementioned observations regarding the glucocorticoids mometasone furoate and betamethasone valerate (BMV), suggesting that the *in vitro* permeation test (IVPT) might be more sensitive than the vasoconstrictor (VC) assay, this study was undertaken to evaluate the use of IVPT a possible surrogate for the determination of glucocorticoid BA/BE. The pharmacokinetics of clobetasol propionate (CP) absorption from five commercial products was assessed in the *in vitro* model and the results compared to those obtained by VC assay using the same products so that a direct comparison of the results obtained by the two methods could be undertaken. It should be emphasized that the primary focus of the study was on BA, not BE. The sensitivity of the two methods was to be evaluated on the basis of their ability to detect differences in relative BA between five different CP products, as opposed to the same product from five different manufacturers. Relative BA was assessed directly with IVPT by quantitatively measuring both the rate and extent of absorption over a 48-hour period. Relative BA was assessed indirectly with the VC assay by quantitatively measuring blanching potency, differences between products being an indicator of differences in BA. The resulting data would logically have implications for the use of IVPT in determining glucocorticoid BE as well.

MATERIALS AND METHODS

Materials

The CP products tested included the Temovate® Ointment, Cream, Emollient Cream, Gel, and Scalp Application (all at 0.05%) manufactured by the innovator company, Glaxo Wellcome. Betamethasone valerate solution 0.1% (E. Fougera® and Co.) was included in the VC assay as a potency control. Radioactive water (^3H_2O, specific activity 1.0 mCi/ml) was obtained from New England Nuclear Corporation (Boston, MA). It was diluted in distilled-deionized water to make a stock solution ~ 0.5 μCi/ml.

In vitro Percutaneous Absorption

Cryopreserved, dermatomed (~0.5 mm) human cadaver skin, all from the posterior trunk, was obtained from a skin bank and stored in water-impermeable plastic bags at −70°C until the day of the experiment. Immediately prior to use it was thawed by placing the bag in 37°C water, then rinsed in tap water to remove any adherent material from the surface. Skin from a single donor was cut into multiple sections large enough to fit on 0.8 cm^2 Franz Cells (PermeGear Inc, Hellertown, PA). The dermal chamber was filled with phosphate-buffered saline (PBS), pH 7.4, and the epidermal chamber left open to the ambient laboratory environment (20–22°C, 35–55% RH). The cells were then placed in a diffusion apparatus in which the dermal receptor solution was stirred magnetically at 600 RPM and its temperature controlled at approximately 36°C to maintain the skin surface at 32° ± 1.0°C.

To assure barrier integrity of each skin section, its permeability to ^3H_2O was determined before application of the test products. Following a brief (0.5–1 hour) equilibrium period, ^3H_2O was layered across the top of the skin by dropper so that the entire exposed surface was covered (~0.2 ml). After 5 minutes the aqueous layer was removed and the skin surface carefully blotted dry. At 30 minutes after application the receptor solution was collected and analyzed for radioactive content by liquid scintillation spectrometry. Skin specimens in which absorption of ^3H_2O was <1.25 μL were considered acceptable. Sections failing this criterion were either discarded

or used as non-dosed blank analytical control chambers. Two to four sections per donor were used for each CP product.

Following the integrity test the receptor solution was changed several times to remove all traces of radioactivity and then replaced with a 1:10 dilution of PBS. (This was done because all receptor samples required concentrating to quantify CP and a high salt content interfered with the analytical procedure.) Subsequently, the chimney portion of the chamber was removed to facilitate access to the skin surface and the four semi-solid products applied using the rounded end of a thin glass rod at a target dose of five milligrams. The applied dose was determined by weighing the rod before and after application. Temovate Scalp Application (a solution) was applied using a positive displacement pipette (Eppendorf Repeater Pipette) at a dose of five microliters. With all products care was taken to evenly distribute the dose over the entire 0.8 cm^2 available application area.

Five to ten minutes following dose application the chimney was replaced and the chamber inspected to ensure no air bubbles had developed on the underside of the skin during the dosing procedure. At 4, 8, 12, 24 and 48 hours the receptor solution was removed in its entirety and replaced with fresh solution, to maintain sink conditions, and an aliquot taken for drug assay by high performance liquid chromatography (HPLC). The applied CP product remained on the skin for the entire 48-hour period. Since the number of acceptable sections from each donor skin was not sufficient to evaluate all products simultaneously on the same donor, the products were run in sets with the cream product serving as the control for all but one donor.

Analytical Methods

All samples were assayed for CP content by HPLC. A 4.0 ml aliquot of each receptor fluid was concentrated by vacuum-drying (Savant SpeedVac, Farmingdale NY) then redissolved by the sequential addition of 50 μL water and 50 μL methanol. Following vortex mixing and centrifugation at 13,000 rpm for 10 minutes, the clear supernatant was transferred to an HPLC small volume insert for analysis. Chromatography was performed on a Hewlett-Packard 1090 M HPLC System. A solvent consisting of methanol and water (75/25 v/v) was maintained at 0.5 ml/min at 40°C through a reverse phase C18 (3 μ) 100x4.6 mm, Luna column (Phenomenex, Torrance CA). Eluting peaks were monitored at 240 nm referenced to 450 nm using a diode-array detector.

Data Analysis

The applied dose was calculated on the basis of product labeled strength (0.5 mg/g) times the mass applied. The CP dose calculated for the solution product (Scalp Application), which was applied by volume not weight, was adjusted for density (measured at 0.91 g/ml). The rate of absorption (ng/cm^2/h) was calculated by dividing the amount of CP absorbed during each sampling interval by the duration of the interval in hours and adjusted for the area of application (0.8 cm^2). Total absorption was calculated as % of applied dose rather than ng/cm^2 since application of the four semisolid products resulted in the applied dose varying slightly from cell to cell. It was obtained from the sum of CP content in all receptor samples divided by the applied CP dose. In both cases the data from replicate skin sections were first averaged to determine the mean value per donor per product, then the mean ± standard error for each product determined by averaging across all donors.

The mean rate of absorption, plotted at the mid-point of the sampling interval, is presented graphically without further analysis. Total absorption is presented both as that obtained from all donors, ignoring the fact that not all products were evaluated in all donors; and also as a ratio in which each product is compared with the cream product to determine relative BA using only those donors in which the two were run concurrently. Pair wise comparisons of the ratios were made using ANOVA followed by the Newman-Keuls method adjusted for unequal sample size [8]. Significant differences were evaluated using alpha = 0.05.

In vivo Vasoconstrictor Assay

This study followed the tenets of the Declaration of Helsinki promulgated in 1964 and was conducted with institutional review board approval. Male and female subjects between 18 and 65 years of age who met all inclusion/exclusion criteria as stated in the FDA Guidance [7] describing use of the vasoconstrictor assay for BE testing, and gave informed consent, were prescreened to determine their ability to exhibit blanching following CP application. Ten microliters of 0.05% CP ointment were applied to a 3.14 cm^2 site on the inner aspect of the ventral forearm using a positive displacement pipette. The dose was spread evenly over the entire area with the Teflon tip of the pipette. After 3-hours the site was wiped three times with dry cotton swabs and 2-hours later skin blanching was assessed visually. Based on the following 0–4 scale, only those subjects exhibiting a reaction≥1 were entered into the study.

0 = no pallor, no change from surrounding area
1 = mild pallor, slight or indistinct outline of application site
2 = moderate pallor, discernible outline of ~ 1/2 application site
3 = moderate pallor, distinct outline of application site
4 = intense pallor, distinct outline of application site.

Forty-eight subjects fulfilled the prescreening requirements and were dosed with the test products. Subjects were instructed to shower or bathe the evening before or at least

2 hours before reporting to the study facility and not bathe during the course of the study. They were also instructed to refrain from using emollient creams or topical products on the forearms for 24-hours before and throughout the study. Eight 3.14 cm^2 sites were marked on the ventral forearm with an indelible marker using a prepared template. The template assured that all sites were within an anatomical area that was a minimum of 5 cm above the wrist and 5 cm below the antecubital fossa, and that all sites were at least 1 cm apart (edge to edge). An additional two sites were marked on the lower portion of the upper arm to serve as untreated control sites.

The six test products, five CP and the BMV control, were randomly assigned to one of eight test sites. (Two additional research formulations were included to make a total of eight products, but their data are not reported here.) A 10 μL quantity of product was applied and evenly spread over the entire site using the rounded end of a small glass rod previously shown to leave >98% of the dispensed drug on the skin. A non-occlusive guard was placed over the forearm to prevent accidental smearing or loss of product to clothing or hands. Three hours after dose application the residual formulation was removed from the sites by wiping with three dry cotton swabs.

A three hour dose-duration was chosen to allow a maximum blanching response (E_{max}) to be achieved. Studies conducted in this laboratory as well as data from other laboratories indicate that 3 hours is more than sufficient for this purpose [9–15]. A maximum response was chosen since the objective of the study was to correlate blanching data with permeation data in which total absorption is the prime parameter for comparison. It should be noted that this is a distinctly different use of the method outlined in the VC guidance in which products are compared at half-maximal blanching (ED_{50}) to avoid the possibility that the capacity of the microvasculature to respond might be exceeded.

Skin blanching was assessed at baseline (0.5–1 hour prior to dosing) and at 1, 2, 3, 4, 8, 12, 24, and 30 hours post-dose removal using a Minolta Chroma Meter (CR-300, Ramsey, NJ) programmed to collect three replicate readings using the L*a*b* color space scale. A rubber O-ring (3 cm ID, 3 mm thick) was attached with double-sided tape to the surface of the sensing head of the chromameter to prevent direct contact between sensor and skin. When taking a reading, the instrument was hand-held by the operator and the O-ring lightly placed onto the skin surface, to avoid compression of superficial blood vessels. The instrument was maintained perpendicular to the skin surface for all readings. All post-dosing a* scale chromameter data were corrected by subtracting the baseline value for that site, as well as subtracting the average change from baseline for the two untreated control sites at the same assessment time. The adjusted a* scale values from all subjects were averaged to give a mean per product value at each time point, and the area-under-the-effect curve from time 0 to 30 hours ($AUEC_{0–30}$) calculated using the trapezoidal rule.

RESULTS

In vitro Permeation Test

A total of 124 skin sections obtained from the posterior trunk of fourteen donors were used to assess CP absorption from the five test products. The average rate of absorption from each product is presented in Fig. 1. The overall absorption profile is similar among the five products and is characterized by a rise to a broad peak in which the maximum rate occurs between 10 and 18 hours. However, large differences between formulations in the magnitude of the peak exist, with the ointment product being more than 10-fold greater than that of the emollient cream.

A summary of the data showing total drug absorption at 48 hours, expressed as percent of applied dose, is presented in Table 1. Absorption varied from a low of 4.3% of the dose for the emollient cream to a high of 62.3% of the dose for the ointment. Relative BA was calculated using the cream product as the comparator and assigning it a value of one. This latter calculation utilized data from only those donors in which there was a direct head-to-head comparison with the cream product, in contrast to the calculation of average total absorption where data from all donors were utilized. Statistical analysis of relative BA showed that CP absorption from the ointment was significantly different from all other products and that absorption from the gel and cream were significantly different from the ointment, solution and emollient cream products.

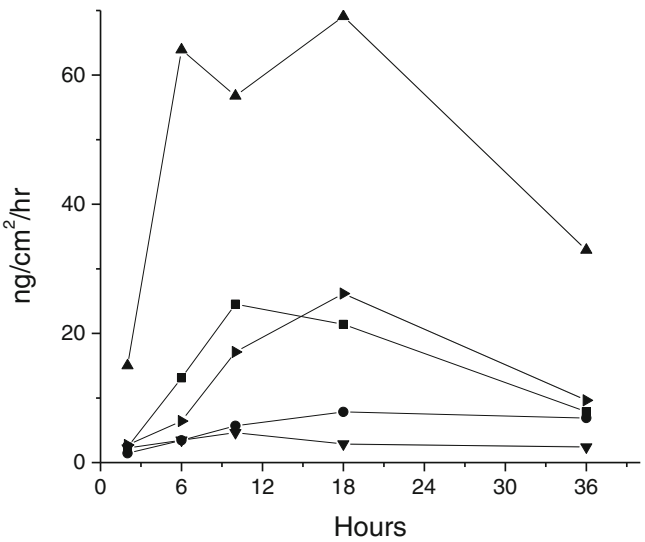

Fig. 1 Rate of CP absorption from Ointment (▲), Gel (▶), Cream (■), Solution (●), and Emollient Cream (▼).

In vivo Vasoconstrictor Assay

Forty-eight subjects entered and completed the study. However, two subjects failed to show any blanching response to all products, in spite of the fact that they exhibited blanching when prescreened (a not uncommon finding in the conduct of the VC assay), and their data were not included in the analysis. Mean skin blanching data are presented in Table 2 and the time course of blanching is presented in Fig. 2.

All five CP products elicited a much greater blanching response than that of BMV lotion, demonstrating that the subject population under study had the sensitivity to discriminate between products known to be of different potency as well as therapeutic effectiveness. In one respect the results tended to parallel those of the permeation study in that the CP products with a high rate of absorption (ointment, cream, gel) elicited a numerically greater blanching response than those with a low rate of absorption (emollient cream, solution), though the rank order for the emollient cream and solution was reversed from that seen with IVPT. However, differences in AUEC between products were small and the variability quite high, resulting in statistical significance only being attained between the ointment and solution products as well as between the cream and solution products. There was no difference between CP cream and ointment as was seen with IVPT.

Inspection of the time course of blanching given in Fig. 2 suggests that it is closely linked to the rate of CP absorption when compared to the flux data presented in Fig. 1. Maximum reduction in the a* value occurs between 10 and 15 hours for all five products, the time at which the CP flux is either at its maximum or is close to its maximum value. This is then followed by a decline in blanching that parallels the declining *in vitro* flux. The parallel between the two time courses can be seen most clearly by comparing the data obtained with the cream and solution products, because of their contrasting absorption and blanching kinetics, and by plotting the blanching response as a positive rather than negative value (Fig. 3). The time lag in the appearance of blanching is presumed to reflect the time for a pharmacologically active concentration of CP to be attained in the dermis since McKenzie has reported that intradermal injection of glucocorticoids elicits blanching within 60–90 minutes [16].

The parallel between the rate of absorption and blanching profiles may at first seem puzzling in that the CP dose is removed after 3 hours in the VC assay but not in the permeation test. However, this finding is entirely consistent with theoretical expectations based on diffusion from a finite dose [17–19]. The time at which the maximum rate of absorption occurs is largely the result of only two factors, the diffusion coefficient and stratum corneum thickness. The size of the applied dose has very little effect. Thus, partial removal of the dose at 3 hours with surface cleansing can reduce the amount of drug absorbed, but the time course will remain essentially the same. A comparison of the blanching response of the ointment and solution products illustrate the point. The solution contains only isopropyl alcohol and water as the solvent system and dries within a matter of minutes. After 3 hours it is unlikely that three dry cotton swabs are capable of removing a significant amount of drug. The ointment product, however, remains essentially unchanged after 3 hours and is easily removed by the dry swabs; yet, a comparison of the blanching response shows the maximum response to occur at the same time for both products (Fig. 2).

Table I Summary of CP *in vitro* permeation data

Product	# Skin Sections/# Donors	Total absorbed (% dose/48 hr)	Relative BA
Ointment	12/4	62.3 ± 9.0	3.3 ± 0.4[a]
Gel	12/4	20.8 ± 3.6	1.5 ± 0.2[b]
Cream	50/13	18.0 ± 1.7	1.00[b]
Solution	30/6	10.3 ± 1.7	0.52 ± 0.1
Emollient Cream	20/6	4.3 ± 0.7	0.31 ± 0.1

Data presented as Mean ± SE

[a] Significantly different from all other products

[b] Significantly different from ointment, solution, and emollient cream

DISCUSSION

Given that BA is generally defined in terms of the rate and extent of absorption of a drug to its site of action, it is reasonable to accept that IVPT would be considered as a surrogate to evaluate the BA/BE of topical drug products since, at its essence, it is a test that measures the rate and extent of percutaneous absorption under well controlled laboratory conditions. Furthermore, the literature of the past 40+ years is replete with studies illustrating the sensitivity of the method to determine quantitative differences in percutaneous absorption caused by changes in the composition of the vehicle, a critical factor affecting BA and BE. Although in its most common mode of application IVPT assesses only absorption of the API through the skin, as was done in this study, rather than API penetration to its site of action within the skin, it is not unreasonable to assume that differences in absorption are reflective of differences in penetration since both are limited by passage through the stratum corneum barrier. Therefore, IVPT should yield data that, if not a direct measure of local BA, are at least an indirect measure of local BA.

Comparison of IVPT and VC Assay

In this study IVPT was used to determine the relative BA of 0.05% CP from five marketed products and the results

Table 2 Summary of CP vasoconstriction (VC) data. for comparison the variability observed in the *in vitro* permeation test (IVPT) is also given

Product	AUEC	CV (%) VC Assay	CV (%) IVPT
Ointment	$-28.6 \pm 3.5^{a,b}$	82	30
Cream	$-26.8 \pm 3.1^{a,b}$	78	35
Gel	-23.4 ± 2.9^{a}	84	36
Emollient Cream	-21.1 ± 3.1^{a}	100	43
Solution	-17.4 ± 3.2^{a}	126	39
BMV Lotion	-5.3 ± 3.0		

Data presented as mean ± SE

AUEC, area under the effect (blanching) curve, *CV*, coefficient of variation

[a] Significantly different from BMV Lotion

[b] Significantly different from solution (Scalp Application)

compared to those obtained using the VC assay. The results illustrate a striking disparity in the sensitivity of the two tests. Whereas the greatest difference between any of the five products was only 1.6-fold by VC assay (ointment *vs* solution), IVPT found a 6.6-fold difference between the same two products and a 10-fold difference between the ointment and emollient cream. A similar finding was obtained by Harding *et al.* in a study conducted on four CP formulations [20]. Large differences in the plasma levels of parent drug were observed following application to volunteer subjects, but these differences were barely detectable when skin blanching was measured. For example, a >400% difference in the CP plasma level between two cream formulations translated to a <25% difference in skin blanching.

Another noteworthy difference between IVPT and the VC assay is the greater variability observed with the latter. The coefficient of variation for the five CP products ranged from

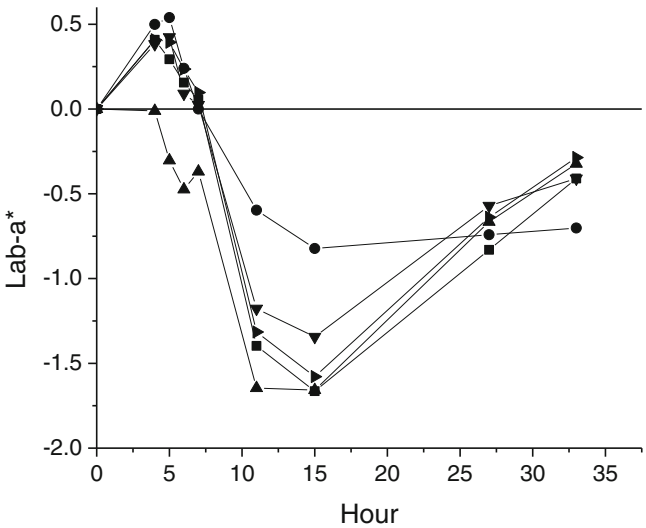

Fig. 2 Vasoconstrictor response measured as change in mean corrected a* scale following product application at T = 0. Ointment (▲), Gel (▶), Cream (■), Solution (●), and Emollient Cream (▼).

78–126% in the VC assay, but only 30–43% for IVPT. As a result, the VC assay could only find a statistically significant difference between the solution and both the ointment and cream products, but could not distinguish the ointment from the cream. In contrast, IVPT could statistically separate the products into three groups: [1] ointment, [2] cream and gel, [3] solution and emollient cream. The results make it quite clear that IVPT is a substantially more sensitive and less variable test than the VC assay at detecting differences in BA between these five products.

The VC results obtained in this study are in agreement with data submitted to the FDA in a number of New Drug Applications available under the Freedom-of-Information Act.[21–24] Table 3 shows the results of four studies, all run by one of the originators of the VC assay (R. B. Stoughton), in which a comparison of one or more CP products with CP cream is made. These studies were conducted prior to issuance of the 1995 BE guidance for topical corticosteroids and, as a result, utilized a different protocol from that employed here. All studies consisted of a 16-hour application of the products to 30 subjects with a single visual reading taken at 18-hours or multiple visual readings taken at 17–30 hours. However, one crucial element common to both the Stoughton protocol and that of this study was the intent to elicit a maximum blanching response. With the exception of CP solution, all products appeared to give a similar blanching response, though no statistical analysis was presented. The insensitivity of the assay is again evident by noting that the cream is <1.2 times more potent than the solution, whereas IVPT shows the difference to be two-fold. Largely as a result of the VC data submitted in these NDAs, the FDA judged the ointment, gel, cream, and emollient cream to be equipotent and labelled them all as "super-high potency"; CP solution is labeled only as "potent" or "highly potent" [25]. This conclusion is not supported by IVPT data.

Could the results of this study have been due simply to differences in the protocol of the two surrogate methods, specifically removal of the dose at three hours in the VC assay *versus* no dose removal in the permeation test? This is highly unlikely since it has been shown previously by others that the blanching response to CP does not increase with application times greater than three hours [9–15].

Saturation of the VC Response

In addition to a higher degree of variability relative to IVPT, the lower sensitivity of the pharmacodynamic response exhibited in this study appears to be a result of saturation of the blanching response. This is shown in Fig. 4 where negative AUEC is plotted as a function of the average rate of absorption at 10 hours, representing the sampling interval (8–12 hours) that best approximates maximum blanching. The

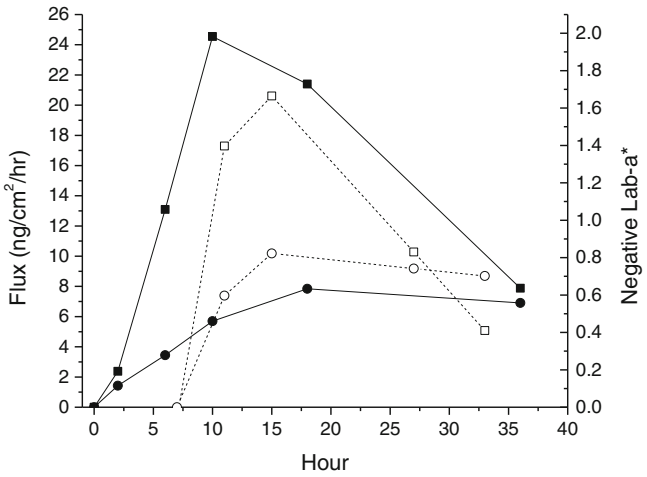

Fig. 3 Comparison of the rate of CP absorption (solid symbols) with the time course of vasoconstriction (open symbols) for the cream (■□) and solution (●○) products.

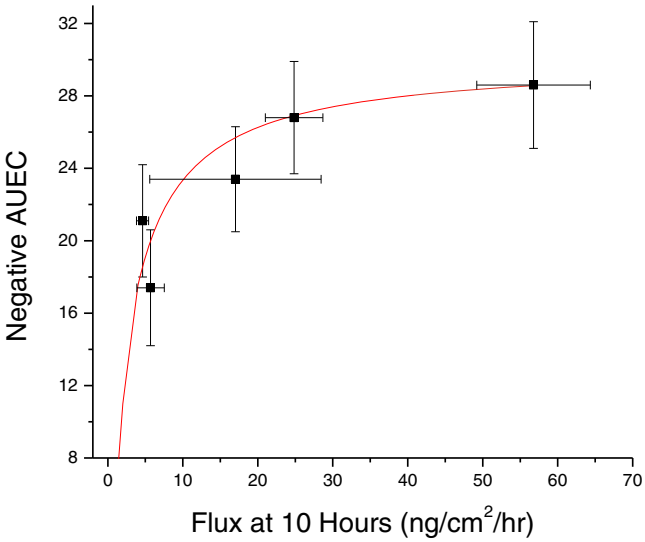

Fig. 4 Negative AUEC *versus* rate of CP absorption for the time interval 8–12 hours. Solid line represents the best fit of the data using the E-max model. (Mean ± SE).

data were fit using the nonlinear dose–response E_{max} model as recommended in the FDA guidance:

$$E_{\max} = E_o + \frac{E_{\max}\ x\ D}{ED_{50} + D}$$

The model describes the measure of an effect (E), in this case as AUEC, in terms of a baseline effect (E_0), a maximal effect (E_{max}), and a dose (D) at which the effect is half-maximal (ED_{50}). The dose in this case is taken as the average flux at 10 hours. As can be seen in the figure, the capacity of the the vasculature to respond at a CP flux >15 ng/cm^2/hr is extremely limited.

Evidence to suggest saturation of the blanching response has been reported before. Wiedersberg *et al.* studied a series of test vehicles containing increasing concentrations of BMV to determine the relationship between the vasoconstrictor response and changes in stratum corneum drug levels [26]. At low BMV concentrations both the level of drug in the barrier, as assessed by tape stripping, and the blanching response increased as the drug concentration increased; but at higher concentrations the blanching response plateaued even though drug levels in the barrier continued to increase. However, there was no attempt to confirm the presence of an increased flux associated with the increase in barrier drug levels.BA *versus* BE.

Table 3 Relative vasoconstrictor potency (CP cream = 1.00) following a 16-hour application as determined by visual grading (0–3 scale)

Product	NDA20340	NDA20337	NDA19968	NDA19966
Ointment			1.00	0.95
Gel		1.02		
Cream	1.00	1.00	1.00	1.00
Solution				0.86
Emollient Cream	0.98			

Data taken from four new drug applications (NDA)

The limitation of any pharmacodynamic assay is evident in this study and points out the need to look historically at the evolution of the VC assay in topical drug development and to differentiate its initial use as a screen for glucocorticoid potency from subsequent use as both: 1) a surrogate for BA in the development of enhanced vehicles, and 2) its regulatory use as surrogate to establish the BE of generic glucocorticoid products. As summarized by Stoughton [27], the search for glucocorticoids more potent than hydrocortisone was greatly simplified by the VC assay since it enabled a direct comparison of large numbers of structural analogs to be made quite simply within a 24-hour time period. Serial dilutions of various analogs in alcohol (avoiding the need for formulation development) were applied to the forearms of human volunteers, left for approximately 16 hours, and the presence of blanching assessed several hours later. The analog that induced blanching at the highest dilution was judged to be the most potent. Since this test was a measure of a drug's intrinsic potency as well as percutaneous absorption, its relevance to the selection of a more efficacious API is clear and the limitation imposed by saturation of receptors was not encountered.

However, the VC assay was subsequently used as part of the formulation development process to select vehicles that would maximize BA of new APIs. In this case the method has a fundamental flaw. Since the blanching response is saturable, vehicles of high BA might be indistinguishable from vehicles of lower BA if saturation is already achieved at a lower level. This shortcoming is amplified by the high degree of variability inherent to the VC assay, and the combined effect of both factors in limiting the sensitivity of the assay is exemplified by the data obtained in this study. Accurate assessment of the BA

of CP products and, possibly, other of the super-potent glucocorticoid products is not possible by VC assay.

Current use of the assay is principally as a surrogate for clinical tests to evaluate the BE of generic glucocorticoid products. In this case the assay is not limited by receptor saturation since the comparison is between two products of near identical composition and the demonstration of equal potency is assessed at half-maximal blanching [7]. This method of conducting the VC assay is quite distinct from that used in the earlier studies of Stoughton where products of divergent vehicle composition were compared at E_{max} [21–24]. It was these studies, unintentionally limited by receptor saturation, that led to the conclusion that CP ointment, cream, gel, and emollient cream are equipotent; a conclusion implying that all should have equal efficacy and safety profiles. This conclusion now seems highly suspect. Direct measurement of "*in vitro* BA" by IVPT concludes that the four products are not equally bioavailable and suggests that they may not be equally efficacious nor have an equivalent safety profile.

Clinical Implications

Differences in the discriminatory power of permeation and vasoconstrictor data have been noted before and, in at least one case, have definite clinical implications. In a clinical trial comparing two marketed BMV products, foam *versus* lotion, the foam formulation was found to be 50% more efficacious in the treatment of scalp psoriasis [5]. The VC assay could find no difference between the two products [28], yet IVPT found a 3-fold greater rate of BMV absorption from the foam formulation, as would be expected given its remarkably greater efficacy [5]. Another study of two marketed products, alclometasone cream and ointment, demonstrated an order of magnitude greater absorption from the ointment product [4]. Yet, the same study found them to be equipotent by VC assay, in agreement with published data showing both products ranked as Class VI steroids in the 7-point classification scheme of Stoughton [27]. No comparative efficacy data are available to define the clinical significance of this finding.

Evidence of the limitation of the VC assay can also be found in a study by Cornell and Stoughton, a study frequently cited as substantiating its value and relevance because of its extensive assessment of clinical correlation [29]. In a series of blinded, paired comparison studies involving application of two different products to symmetrical lesions on the trunk or extremities of psoriatic subjects, they found the efficacy results in 20 of 23 comparisons to be in line with the results obtained by VC assay. However, in three cases the results did not agree and in each case the efficacy of one product *versus* the comparator was less than predicted by the VC assay. This is consistent with the BMV foam *versus* lotion data cited earlier in which the lotion produced an equal blanching response but failed to perform equally in the clinic [5].

The lack of clinical data in which there was a direct comparison of any two of the five CP products tested in this study makes it difficult to evaluate the clinical implications of the large differences in absorption that were observed here.

However, data submitted in two NDAs in which the gel was found to be twice as effective as the emollient cream do suggest that a positive correlation exists [23, 24]. Though the data come from two separate placebo-controlled studies, and not a head-to-head comparison of the products in the same study, both studies were conducted under identical protocols and used the same grading system. At the end of a two week treatment period, 53% *versus* 24% of subjects were rated as clear or >75% improvement for the gel and emollient cream, respectively. Following another two week period with no treatment, the values were 54 and 22%, respectively.

The disparity between absorption data and the blanching response also suggests that differences in the potential to cause systemic toxicity, namely adrenal suppression, may not be detectable by VC assay. Again, the lack of clinical data makes this difficult to assess. Only two studies could be found in which there was a direct comparison of two CP products that differed greatly in absorption, and neither was sufficiently powered to determine statistically significant differences. For example, in a randomized crossover study of 12 subjects with either eczema or psoriasis involving 15–30% body surface area, CP cream was compared to CP emollient cream following one week of treatment with 1.5 grams twice daily [24]. During the treatment period at least one subnormal cortisol level was found in 6/12 (50%) subjects receiving the cream but only 3/12 (25%) receiving the emollient cream.

Similar results were found in a second study comparing the same two products in parallel groups of adolescent patients with atopic eczema, this time using injection of synthetic adrenocorticotropic hormone (Cortrosyn®) to test adrenal function [30]. Following two weeks of treatment, only 1/10 (10%) using the emollient cream *versus* 3/12 (25%) using the cream product exhibited adrenal suppression. Though the results of both studies are in agreement, and consistent with the observed lower rate of CP absorption from the emollient cream, the small number of subjects tested makes it impossible to accept this as definitive proof that greater CP absorption from the cream product results in greater systemic toxicity.

CONCLUSIONS

A comparison of two surrogate tests has revealed that the use of the IVPT to quantify differences in relative BA between five marketed clobetasol propionate products provides a much greater level of sensitivity than that afforded by the VC assay. The permeation test found total clobetasol absorption from the

five products to vary over a ten-fold range whereas the vasoconstrictor assay found this same difference was less than two-fold. The discriminating power of vasoconstriction was constrained by much higher variability as well as apparent saturation of the response at the high levels of clobetasol absorption exhibited by these products. In agreement with earlier studies, these data continue to support use of IVPT for the determination of relative BA and "*in vitro*" BE of many, if not all, topical products. Presently, the new data specifically argue for its acceptance as a valid, alternative surrogate to establish the BE of topical glucocorticoid products.

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