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The Human Skin Blanching Assay as an Indicator of Topical Corticosteroid Bioavailability and Potency: An Update

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The human skin-blanching (or vasoconstrictor) assay has evolved from initial observations that corticosteroids induce a pallor or whitening of the skin to which they are applied. McKenzie and Stoughton (1962) are generally recognized as having developed the first scientific bioassay for comparing corticosteroid potency. The extensive use of this bioassay to compare drug release from topical delivery systems has demonstrated numerous instances in which the topical bioavailability may vary greatly, dependent on the character of the delivery vehicle. It has become evident that simply incorporating an intrinsically potent drug into a formulation does not necessarily produce a clinically efficacious product.

The traditional definition of bioavailability refers to the rate and extent of drug appearance in the systemic circulation following administration of the dosage form. However, for topically applied drugs the monitoring of serum concentrations would indicate the rate of drug removal from the pharmacological site of action. It may, therefore, be more appropriate to define topical bioavailability in terms of the ratio between the amount of a specific drug that is applied to the skin and the amount that reaches the active sites. Such a definition would encompass parameters of drug release from the applied vehicle, partitioning into the stratum corneum and diffusion through this layer. In terms of the human blanching assay, bioavailability may be used to refer to the relative absorption efficiency of the same drug from different vehicles, as assessed by the intensity of the induced blanching. Bioavailability and intrinsic activity

are not synonymous; the latter indicates the clinical efficacy of the drug (and its ability to induce skin pallor) while the former refers to the activity of a specific corticosteroid in a given vehicle compared to its activity in a different base.

Since the initial experiments of McKenzie and Stoughton, several research groups have developed variations of this initial procedure for assessing and comparing topical drug bioavailability (Falconi and Rossi, 1972; Place et al., 1970). The assay has been used, for example, to compare drug release from ointments (Barry and Woodford, 1975), creams and gels (Barry and Woodford, 1974), and for the comparison of trade name products to generic equivalents (Stoughton, 1987). With this multitude of experimental methodologies, problems arise in attempting to compare results from different investigators or in assessing the validity of conclusions drawn from certain experimental protocols. Haigh and Kanfer (1984) have published a detailed blanching trial procedure that attempts to minimize many of the variables and permutations common to the methodologies of other researchers. Their report specifically addresses issues such as the recording of a single *versus* multiple observations, the optimal contact time of the drug with the skin, and the investigation of occlusive and nonocclusive application conditions on the magnitude of the induced blanching. This optimized experimental methodology has been demonstrated to be a sensitive, accurate, and reproducible technique for comparing the bioavailability and potency of topical corticosteroids (Barry and Woodford, 1978; Haigh and Kanfer, 1984).

GENERAL BLANCHING TRIAL METHODOLOGY FOR TOPICAL DOSAGE FORMS

The assay procedure reported by Haigh and Kanfer employs 12 healthy, Caucasian men and women who have not received corticoids, either systemically or topically, for at least 6 weeks before the study. Blanching is difficult to discern on highly pigmented or tanned skin, presumably because the melanocytes obscure the underlying vasculature, and it is barely visible on black skin, even when exposed to potent, fluorinated corticoids (Haigh, Meyer, and Smith, unpublished observations).

Adhesive labels, from which two 7 X 7-mm squares have been punched, are applied to the flexor aspects of both forearms of each volunteer, avoiding hirsute areas whenever possible. Usually 12 discrete application sites are demarcated along the length of each forearm in this fashion. Topical formulations to be evaluated are applied to these sites either by extrusion from a 1-ml syringe (with the needle cut to 5 mm to facilitate extrusion), or by the use of micropipettes for fluid preparations. The extruded formulations are spread evenly over the application sites by the use of glass rods. Usually, four application patterns are devised, and one of the patterns is randomly assigned to each arm of each volunteer to prevent the appearance of a recognizable blanching pattern that may occur if the same pattern was used for each volunteer. Similarly, the

preparations are coded before application, maintaining the double-blind nature of the investigation. One arm of each volunteer, usually the dominant arm, has the spread preparations on the application sites covered with strips of impervious tape ("occluded sites"), thereby preventing evaporation of moisture. The dominant arm of each volunteer is usually chosen for occlusion in this manner so that normal movement (writing for example) is impaired as little as possible during the exposure period. The sites of the other arm are covered with a porous Perspex or cardboard frame that will prevent accidental removal of the applied formulations by abrasion with clothing but will not prevent escape of moisture to the atmosphere ("unoccluded sites").

The formulations remain in contact with the skin for 6 h, after which the guards, occlusive strips, and demarcating labels are carefully removed. Residual formulation is gently washed from the sites with soap and warm water, and the skin patted dry with a towel. The puckering of the skin, due to hydration, and slight erythema that results from adhesive tape removal usually subsides within 30 min. Thereafter, three trained observers independently assess the degree of induced blanching at each site at regular intervals. Observations are typically made at 7, 8, 9, 10, 12, 14, 16, 18, 28, and 32 h after initial application. Standard overhead fluorescent lighting is used to illuminate the horizontally placed arms of the volunteers, flexor aspect uppermost.

The usual method of recording the blanching involves the subjective assignment of a number between 0 and 4 that represents the perceived intensity of blanching at each site (0 representing normal skin, 4 representing intense blanching with distinct edges, and 1-3 representing intermediate grades of blanching). The independent observation data from the three observers are usually summated, after appropriate decoding, and used to generate two blanching versus time profiles over the observation period (occluded and unoccluded data). The degree of blanching is usually expressed as percentage of total possible score (%TPS), calculated from the quotient of the actual score (AS) and total possible score (TPS). The TPS is the product of the maximum possible score per site (usually 4 for fluorinated corticoids), number of independent observers (usually 3), the number of sites per preparation per arm (e.g., if three preparations are compared, each will be applied to 4 of the 12 sites on each arm), and the number of volunteers (usually 10-12). The AS equals the sum of the frequencies of the graded responses recorded for each preparation at each site and the %TPS is given by (AS/TPS) $\times$ 100. The generation of blanching profiles in this manner allows calculation of an area under the curve (AUC) value by standard trapezoidal summation. A "bioavailability" curve is thus produced for each preparation in each application mode and allows comparative examination of aspects such as the peak blanching elicited, time to peak blanching, and duration of blanching. Normal statistical analysis may be applied to the results to examine the significance of the difference between profiles.

Other methods of recording the degree of blanching include a simple yes/no observation of vasoconstriction at each site or a direct greater-than or less-than comparison of blanching at adjacent sites (Raymond et al., 1985), however, these methods are generally considered to be less informative than the full-curve technique.

In this manner, precise and reproducible comparisons may be made of the drug release from two preparations and of relative potencies of the preparations if two different corticosteroids are compared. Usually, drug release from a test formulation is compared with that from a standard or approved product, and often a "reference" formulation is incorporated into every assay to lend credence to the results. It is now generally considered that the degree of blanching elicited in such an assay is a good indication of the clinical efficacy that may be expected from the product in dermatological use (Bluefarb et al., 1976; Burdick, 1972; Cornell and Stoughton, 1985; Gibson, 1985; Haleblian et al., 1977; Smith et al., 1973; Stoughton, 1972, 1976; Whitefield and McKenzie, 1975). Furthermore, Engel et al. (1974) note that assessment by the blanching assay is sufficiently accurate to estimate the structure-activity relationships of topical corticosteroids, and it is preferable to some other techniques. Hence, the human blanching assay appears to be a valuable tool, not only in comparing the bioavailability of a corticosteroid from different delivery systems, but also in estimating the clinical usefulness of a dosage form. However, some aspects of the methodology require special attention to detail.

Volunteer Selection

Certain individuals do not exhibit a discernible vasoconstrictive response to the topical application of corticosteroids, although these individuals are clinically responsive to the drugs when employed in dermatotherapy. It is, therefore, necessary to conduct a preliminary screening of all prospective volunteers so that those subjects who do not exhibit a blanching response may be excluded from the experiment. This may be easily accomplished by the application of a small quantity of corticosteroid cream to the upper arm and occluding the application area with impervious tape. After 6 h the tape is removed, the skin is washed, and at approximately 8 h the site is observed for discernible blanching.

Of the subjects that react positively to this initial screening, a normal biological variance is usually observed: some volunteers blanch strongly and others relatively weakly to the same drug dose. Subjects who blanch strongly are usually poor discriminators in the assessment of comparative blanching between potent corticosteroid formulations. In these persons, each of the products applied to the skin sites, even if dosages are markedly different, may induce maximal blanching and may result in the erroneous conclusion that the products are bioequivalent or equipotent. In contrast, it is usually difficult to discriminate skin blanching from normal skin mottling in subjects that respond poorly to topical

corticosteroids. Alternatively, some subjects' reactions are clearly able to discriminate the different drug release rates from different formulations. In any assay the ideal situation would be to include only good discriminators; however, subjects blanch to different extents at different times and, hence, a spectrum of "reactors" is usually included into each trial group (good, poor, and intermediate blanchers). In this manner a mean blanching response may be calculated when the results are summated.

On the other hand, if a corticoid of inherently low blanching ability (such as hydrocortisone) is to be tested, then it may be better to include only good (and possibly some medium-response blanchers) in the assay. It is, thereby, possible to ensure that blanching will be elicited at sufficient sites to determine the statistical analysis of the results: blanching recorded at all the application sites (even though it may be poor in each case) would produce results of greater statistical validity than conclusions drawn from blanching observed at only 45% of the sites, for example. Equally, if very potent drugs in simple alcoholic solution are to be compared (intense vasoconstriction expected) then poor and intermediate blanchers are more appropriate subjects. Hence, in certain circumstances, the selection of specific volunteer subgroups may be a sensible practice and may produce results of greater validity, while not detracting from the integrity of the assay methodology.

Another factor that may bear consideration in the selection of subjects is the menstrual fluctuations in circulating female hormone levels and the possible influence that these may have on vascular tone and reactivity at different periods within the cycle. Although not a cause for great concern, as all of the formulations are simultaneously compared at any time, it may be appropriate to limit the number of female subjects dependent on the specific details of the study.

Application Site

Several reports are concerned with the differing magnitudes of percutaneous absorption through the skin of different anatomical regions, and the effect that this may have on the blanching response (Feldman and Maibach, 1966; Idson, 1983; Ishihara, 1976; Kidd, 1975; Osamura, 1982; Rougier et al., 1987; Stuttgen, 1976). Initially this variance was thought to be due to differences in the thickness of the stratum corneum at these sites; however, reports by Elias et al. (1980) postulate a definite contribution of the skin lipid composition in determining skin permeability at each site. Careful selection of an appropriate anatomical site for blanching assays must, therefore, be made.

Classically, the inner aspect of the forearm has been used by several researchers, because this site has several advantages: the skin is relatively permeable in this region; little inconvenience is experienced by the volunteers while the tape, guards, and formulations are applied to the forearms; and repeated removal of clothing for observation of blanching is avoided. Furthermore, several dermato-

logical conditions afflict the limbs and, hence, the use of the arm as an experimental site appears justified and appropriate in that this may be the target for eventual clinical use of the formulation. However, research (Barry and Woodford, 1978; Burdick, 1974; McKenzie and Atkinson, 1964) has indicated that blanching inconsistencies, or a blanching "gradient" (Kirsch et al., 1982), may exist between sites at the wrist and elbow after exposure to equivalent doses of corticosteroid. Nevertheless, if the methodology detailed here is adhered to, then the presence of a blanching gradient does not present a problem, for each formulation is applied to several sites along the entire length of the forearm.

If the dosage form is intended for clinical application at a site other than the limbs, then the extent to which healthy forearm skin resembles the epidermal absorptive barrier at these unique sites must be assessed. For example, it has been shown that the skin of the genital region is more permeable than forearm skin to topically applied agents (Feldmann and Maibach, 1966) and, hence, the applicability of results obtained from blanching assays conducted on the limbs to the bioavailability of the drugs when applied to genital skin is within question. It is obviously difficult to test anorectal skin, for example, in this manner and, hence, it is often necessary to make certain assumptions when comparing bioavailability data.

The use of skin from the intended clinical delivery site should not be imperative if comparative investigations are conducted. Two topical products that demonstrate bioequivalence at the forearm application sites may, within reason, be expected to show bioequivalence at other anatomical sites, although the extent of drug absorption may be different in the two regions. Equally, if the drug bioavailability from one product is superior at forearm blanching sites, it may be presumed that drug absorption will differ in a similar manner at other anatomical regions. The single proviso for such an assumption is that all of the formulations tested are manipulated in an identical manner and exposed to identical conditions at the forearm sites, a condition that is fulfilled by the assay methodology detailed earlier.

Similarly, the application of the forearm-blanching assay may indicate a potential for inducing drug toxicity at other sites. If forearm drug bioavailability from a test formulation is superior to that of a standard preparation, then application of the test product to skin of greater permeability may result in significant drug absorption and may produce marked systemic drug concentrations. The potential for systemic or localized iatrogenic manifestations from the long-term use of such a product bears consideration. The researcher must, therefore, determine from the results of the blanching assay if the compared products are bioequivalent and, if superiority of the test formulation is exhibited, the potential for producing toxic drug concentrations at the clincal application site must be evaluated.

An alternative to extrapolating forearm results to those expected at other anatomical sites may be to alter the physicochemical nature of the forearm skin so that its permeability is increased, simulating that of other anatomical regions. Pretreatment of the skin with chemicals or removal of the barrier stratum corneum layer (or part of it) are methods that may be adopted, however, several problems are anticipated with this practice. The extent of pretreatment or stratum corneum removal, required to adequately simulate the permeability of skin from a different region needs careful evaluation. The use of chemical pretreatment (Bucks et al., 1983) introduces a further variable into the already complex hydrophilic-lipophilic biochemical balance of the stratum corneum and, hence, this is not assumed to be a technique of choice.

The removal of stratum corneum cell layers by repeated adhesive tape stripping was first adopted by Wells (1957) and has subsequently been used by several researchers to study the permeation of chemical through "damaged" skin (Bronaugh and Stewart, 1985; Rougier et al., 1987; Washitake et al., 1973). Percutaneous drug bioavailability through stripped skin is anticipated to be markedly greater than that through unstripped skin, and it may even be greater than that through the inherently permeable skin from unique anatomical regions. The fundamental question that must be addressed is whether or not stripped skin, in comparison with healthy forearm skin, more closely simulates the more permeable tissue of another anatomical region.

It is assumed that by reducing the barrier to absorption by removal of stratum corneum layers the ability of the skin to discriminate between drug delivery potentials of two similar formulations may be decreased. There may be a greater tendency for maximal blanching to be elicited at all sites, regardless of small drug-release differences from the formulations. The sensitivity of the blanching assay in this situation is, therefore, reduced. Additionally, the ability to reproducibly strip each skin application site to exactly the same extent is difficult. Even small variations in the thickness of the barrier layer remaining after stripping adjacent sites may result in greatly differing drug absorption rates. Stripping of the stratum corneum would, therefore, introduce further variables into the assay methodology, probably resulting in greater variance in the results and would thus require a greater number of application sites for valid conclusions to be drawn.

The possibility of partial stratum corneum removal should also be addressed. This may theoretically be accomplished by application and removal of a single adhesive tape strip (in contrast to the repeated strippings required to produce the glistening epidermis associated with complete stratum corneum removal). A single stripping, although possibly removing more than half of the superficial cell layers, will leave several lower strata intact, thereby preserving some of the barrier to absorption. In this manner forearm skin permeability may, theoretically, be reduced; however, the question of stripping uniformity is of major

concern. It seems improbable that several sites would be stripped to exactly the same extent for reliable comparisons to be made of the subsequent blanching at these sites.

Generally, it is proposed that the entire process of transdermal drug absorption is too complex and too interactive to presume that removal of the barrier stratum corneum layer from an anatomical region of relatively low permeability would cause the stripped skin to mimic that of more permeable regions. Healthy, unstripped skin presents a uniform resistive environment at all prospective test sites; it does not appear as though this barrier may be uniformly reduced at several sites by adhesive tape stripping.

Although chemical or physical alteration of healthy skin is not advocated for bioequivalence testing, the results from such a procedure may be useful if performed as part of a battery of different bioassays to compare drug release rates. If, for example, drug bioequivalence from two topical products is demonstrated by blanching assays on normal skin, partially and fully stripped skin, and possibly by certain in vitro experiments, then it may be assumed with a fair degree of confidence that the drug-release rates from the two formulations should be similar at all anatomical application sites.

Dosage Form Application

Theoretically, the requirements for the drug application procedure are fairly simple: an equivalent mass or volume of each preparation to be tested must be applied to skin sites of uniform area, preferably in a double-blind manner. It is imperative that the drug dose per unit skin area be identical throughout and that the applied dosage form be contained within a clearly demarcated area for the entire contact period. From an observational viewpoint, it is important that the application sites have definite margins, especially if weakly vasoactive drugs are to be tested, so that the region of blanched skin may be clearly discerned from surrounding, normal skin.

The containment of the dosage form to a specific area may be accomplished in several ways. The most facile appears to be the use of demarcating labels (adhesive patches that have had square holes of uniform dimensions punched from their centers). Alternatively, stainless steel (Finn or Duhring) chambers of specific volume may be used to contain the formulation, however, the use of these chambers preclude the blanching assay being conducted in the unoccluded mode (Kaidbey and Kligman, 1974). It must be borne in mind that these chambers have a relatively large volume/surface area ratio, and even though several milligrams of formulation may be required to fill the chamber, the drug will only be depleted from the formulation laminae juxtaposed to the skin. The size of the dosage employed in the assay, therefore, requires consideration, especially as the use of "excess" formulation in this manner does not generally mimic conditions of clinical use.

Several methods have been adopted by researchers for the application of the delivery vehicle on to the skin test sites. Methods that have found the most favor include the extrusion of semisolid formulations from a small disposable syringe, the use of a spherically tipped glass rod to directly apply and spread the formulation, or the use of a transfer pipette to deliver a finite volume of liquid vehicle. The specific technique adopted in any study does not appear important, provided it has been demonstrated to be precise and reproducible for the volume or mass of the vehicle applied to each site. Generally, some practice is required by the operator to attain proficiency in application uniformity.

For syringe extrusion, Magnus et al. (1980) have applied varying numbers of "stripes" of formulation to the sites and have demonstrated fair precision in this methodology (typically four stripes of cream or ointment of 7-mm length are extruded onto an area of 7 X 7 mm, thereby depositing approximately 3 mg of formulation). Barry and Woodford (1974) have demonstrated that applying between 3 mg and 8 mg of betamethasone 17-valerate formulation in this manner had little effect on the degree of the resultant blanching. However, Magnus et al. (1980) report a proportional increase in blanching when 1.6-4.8 mg of cream was applied to the sites, but no further increase when masses greater than 4.8 mg were applied. It is anticipated that doses in excess of this datum contributed little to maintaining the drug concentration and flux at the stratum corneum interface.

Alternatively, greater precision may be possible by the use of a glass rod, dipped into a reservoir of the formulation, and used to spread the product onto the skin (Barry and Woodford, 1974; Smith, 1987). The size of the spherical rod tip may be varied to deliver different quantities of the vehicle. The use of a transfer pipette appears to be the most appropriate for the application of free-flowing liquids; however, the pipetting of volatile solutions may pose a problem in that evaporation of the solvent will concentrate solutions of the test compound and, thereby, detract from application uniformity.

The viscosity of the drug vehicle may have some effect on the magnitude of the induced blanching. Theoretically, the difference in the viscosities of the dosage forms may affect the rate of drug replenishment at the formulation-skin interface. As drug molecules are absorbed by the stratum corneum, replenishment must occur at the interface by diffusion through the bulk formulation vehicle, if the driving force for passive diffusion into the skin is to be maintained. This intravehicle diffusion and replenishment may be greatly facilitated in relatively nonviscous formulations (maintaining the absorptive driving force) but may be concomitantly more difficult in more viscous matrices (with a resultant decrease in the interfacial drug concentration with time). Skin "exposure" to the drug may, therefore, be greater with nonviscous than with viscous formulations of equal concentration and contact time. Hence, the dose of each formulation type required to produce optimal blanching requires evaluation.

Doses of formulation greater than this datum will not further contribute to the vasoconstriction, but may influence other factors such as the occlusion of the application site.

On the other hand, differences in intravehicle diffusion may not manifest in the relatively short periods of formulation-skin contact practiced in the blanching assay, especially if the prospective permeant has little affinity for the skin (low thermodynamic activity) and its concentration does not deplete significantly at the interface. Here, variations in dosing are not anticipated to cause marked variance in the induced blanching. In either event, it appears that evaluation of an optimal dose is required so that maximal blanching may be induced without the application of gross excess of vehicle to the sites. This evaluation may also help maintain the dose at clinical levels, thereby increasing the relevance of the study.

Occlusion of the Dosage Form

The effects of occluding the delivery vehicle with an impervious material, after application to the skin, has been the subject of considerable research. Occlusion prevents loss of moisture from the skin (and from aqueous delivery vehicles) to the atmosphere. This trapped moisture, either endogenous or exogenous, extensively hydrates the stratum corneum, causing it to swell appreciably (Idson, 1975), and (through complex biochemical mechanism) to, generally, enhance the transport of topically applied drugs through it (McKenzie and Stoughton, 1962; Maibach, 1976). In addition, Vickers (1963) has suggested a definite correlation between skin occlusion and the extent of drug reservoir formation in the stratum corneum after percutaneous absorption. It is generally accepted that occlusion increases drug activity in the greater majority of application conditions, and this situation has been exploited clinically if high concentrations of corticosteroid are required to permeate the stratum corneum. Furthermore, the increased permeability of occluded skin may cause the tissue to mimic the lower absorptive barrier of skin from another anatomical region, and the technique may have a useful application here.

On the other hand, in the blanching assay occlusion may mask the subtle effects that different formulation adjuvants may have on the drug delivery to the skin. Two similar topical formulations that may demonstrate variance in drug bioavailability, when assayed in the unoccluded application mode, may produce fairly similar, although higher, bioavailability when applied under occlusion. Therefore, the human blanching assay is normally conducted in both application modes so that bioavailability results may be compared in the two situations.

Certain anhydrous, lipophilic dosage forms are inherently occlusive and will decrease the rate of transpirational water loss from the skin, even when applied in the unoccluded mode. The application of a thick, nonclinical layer of formu-

lation, although not exposing the skin interface to a greater number of drug molecules, would act in an occlusive manner by preventing moisture loss from the skin surface. It has been suggested that this is why ointment formulations often induce greater blanching than creams of the same drug concentration (Coldman et al., 1971; Haigh and Kanfer, 1984; Stoughton, 1972). However, there is evidence to suggest that the water contained in aqueous dosage forms may be more active than transpirational moisture from the epidermis in hydrating the horny layer under occluded conditions (Smith, 1987; Waaler, 1985). It has been observed that the relative increase in drug permeation in the occluded, compared with the unoccluded, application modes, is markedly less for anhydrous than for aqueous formulations. Anhydrous formulations will not deliver further water to the horny layer when occluded with impervious tape, as is presumed to occur with aqueous dosage forms. There is, therefore, a limit on the degree of stratum corneum hydration that can be induced by the additional occlusion of the lipophilic formulations, and this appears to be less than maximal.

Nevertheless, when corticosteroids of low potency are evaluated, it is usually difficult to discern any blanching of the skin at sites that have not been occluded. Hence, hydrocortisone assays are often conducted only in the occluded mode, and the maximum possible score per site for recording and computational purposes is reduced from 4 to 2. The hydrocortisone-induced blanching is generally so weak that scores higher than 2 are seldom recorded if the usual 0 to 4 scoring scale is used.

Therefore, occlusive application conditions are indicated for the bioavailability assessment of low-potency topical corticosteroids and in situations where the lower barrier potential of hydrated tissue is to be investigated. Although occlusion is usually generated by the application of impervious tape, the use of stainless steel chambers to contain the dosage forms is an additional good method of occluding the application site. Furthermore, possible problems of drug adsorption to impervious adhesive tape are avoided; however, the effects that water contained within the formulations may have on additionally hydrating the barrier layer should not be overlooked. Superior bioavailability demonstrated for an aqueous formulation by this occlusive methodology may be an artifact of the greater degree of stratum corneum hydration induced by the hydrous dosage form at forearm sites.

Occlusion Time

The time that the formulations remain in contact with the skin in either application mode may markedly influence the degree of induced vasoconstriction. Magnus et al. (1980) have compared the effects of occlusion times between 2 and 12 h and have found that blanching increases with occlusion time to a pla-

teau value at 10 h. This period of occlusion may induce maximal hydration of the stratum corneum, under the conditions of the assay, thereby delivering sufficient drug to the dermal vasculature to saturate any vasoconstrictor sites. Longer periods of occlusion, therefore, do not induce greater degrees of blanching. If blanching saturation is approached in this manner, then the ability of the assay to discriminate between the drug-release properties of similar formulations diminishes.

Similarly, if application times are too short, then insufficient drug will reach the vasculature to induce discernible blanching, especially if corticosteroids of low potency are evaluated. Application times of 1 or 2 h are, therefore, presumed to be of limited use in comparing topical corticoid bioavailability. A standard application/occlusion time of 6 h has been adopted by several research groups, as this contact period permits maximal differentiation between the drug release properties of similar formulations. Ideally the products should remain on the skin for 6 h, residual formulation should then be removed, and the observation of induced blanching should commence approximately 1 to 2 h later, and should continue over an extended period in order to obtain sufficient data for the construction of a blanching profile at regular intervals for at least 24 h. However, the occlusion time should be tailored for each specific assay so that blanching is optimized but saturation is not attained.

The Reading of Results

The assessment of the degree of blanching induced at each application site is subjective and is graded on a linear scale, usually by a number of independent observers. Investigation has demonstrated that the results from two trained observers do not deviate significantly from the mean results of three observers (Haigh et al., unpublished observations). This observation suggests, theoretically, that the assay may be carried out by two experienced observers; however, three are preferable because of the assessment subjectivity.

The magnitude of blanching may be influenced by a number of variables, such as ambient temperature and relative humidity, and by physiological factors, such as endogenous hormonal concentrations or postprandial influences on cutaneous vascular tone. Similarly, the differences in the vascularization of the dominant and nondominant arms may affect the degree of blanching induced by topical corticoids, the diurnal variation in lighting may influence the perceived intensity of blanching. Several reflectance (Altmeyer and Zaun, 1976; Dawson et al., 1980; Feather et al., 1982; Ryatt et al., 1982; Zaun and Altmeyer, 1973), thermographic (Aiache et al., 1980), or photographic (Trikam and Morton, 1985) techniques have been developed in an attempt to more quantitatively record the degree of skin blanching at each site. However, although more laborious, these instrumental methods have not been proved

superior to the relatively facile method of visual assessment (Engel et al., 1974; Gibson and Kirsch, 1986), and the latter remains in general worldwide use. The fundamental question that must be addressed with these techniques is whether or not an instrumental method of assessing skin hue is more precise and accurate than using a human "instrument." In both cases a pharmacologic side effect of the drug is being measured and not the drug concentration per se and, hence, it is not clear if a number obtained by an instrument is more meaningful than a number obtained from visual assessment.

Laser Doppler velocimetry is a relatively new technique that may have useful application in this field (Stevenson et al., 1987), especially when combined with reactive hyperemia (Bisgaard et al., 1986). This is a fiberoptic method that measures light reflection from the dermis, the wavelength of which has been Doppler-shifted by a factor proportional to the number and velocity of erythrocytes moving through the vasculature. Normal skin may be compared with blanched skin in this manner to yield a "ratio" of vasoconstriction. However, although the technique appears sensitive for the assessment of vasodilation, precise estimation of vasoconstriction, especially weak blanching, appears problematic (Amantea et al., 1983). Measurement problems are compounded if the laser probe is not fixed to the skin, hindering rapid site-to-site estimations of blood flow. It has been suggested that forced, generalized physiological vasodilation, by the immersion of the lower extremities of the volunteers in warm water for example, may accentuate the blanching response and facilitate monitoring by laser Doppler velocimetry (J. Wilkin, personal communication). This procedure may also be of some use in the visual monitoring of the weak blanching elicited by hydrocortisone.

The number of blanching observations required for reliable prediction of drug bioavailability has been a keenly debated topic. On the one hand, several research groups advocate that repeated readings should be taken over a prolonged period after drug application so that a complete blanching profile and AUC value may be generated from the results (Haigh et al., 1985, 1986; Magnus et al., 1980). The generally accepted protocol for comparing oral or parenteral drug bioavailability, and general scientific technique for that matter, suggests that full curve analysis in this fashion would be the optimal methodology to use. Haigh et al. (1985) caution that blanching profiles may be coincident at certain times and greatly divergent at others. Although the drug delivery from two formulations may be assessed as equivalent at a specific time (when the profiles are coincident), the one product may produce a sharp blanching profile which decreases within hours, whereas the other may produce a relatively blunt profile that persists for a longer period. Clearly, the blanching activities (and, by inference, the relative drug bioavailabilities) are different for the products, but a single-reading blanching assay may not demonstrate this difference.

Although single observations of blanching may be useful and appropriate in specific instances (Gibson, 1985), it is obvious that the rate or extent of drug delivery to the skin cannot be fully characterized by a single observation. However, some researchers maintain that topical bioavailability may be adequately compared by taking relatively few (Raymond et al., 1985), or even single (Cornell and Stoughton, 1985; Stoughton, 1987), observations of induced blanching.

Therefore, the recording of multiple readings over a prolonged time is presumed to be a prerequisite for adequate bioavailability comparisons. This technique would, furthermore, serve to identify dosage forms that augment rapid drug delivery to the vasculature (sharp blanching profiles) in contrast to those that may augment partitioning into and reservoir formation within the stratum corneum (blunt, prolonged profiles). Equally, the respective AUC value obtained for each formulation may be important in comparing bioavailability.

SUMMARY

Despite its relative crudeness when compared with modern instrumental analytical techniques, the human blanching assay remains a rapid and reliable test of in vivo transdermal corticosteroid permeation, and it is routinely practiced in several laboratories worldwide. The assay is attractive in that it is noninflammatory and noninvasive, thereby subjecting the volunteers to minimal discomfort, but it is precise and accurate in predicting corticoid bioavailability and potency. With the ongoing research devoted to monitoring drug concentrations in the stratum corneum and in the systemic circulation after topical administration, the interrelationship between blanching and corticoid exposure may be more fully elucidated and, thereby, a more quantitative bioassay may be developed. Investigations continue in an attempt to make the methodology more precise, especially for the application procedures and for observation of the blanching response. With the modern trend toward the development of better drug delivery systems, it is anticipated that the human blanching assay will remain an invaluable tool in assessing the drug release potential of new corticosteroid formulations, especially proprietary products that cannot be monitored by radioscintillography.

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