The human skin blanching assay for comparing topical corticosteroid availability

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The human skin blanching assay remains in widespread use as a reliable, qualitative, comparative indicator of topical corticosteroid availability and potency. The experimental refinements promulgated by certain researchers in this field have yielded a versatile bioassay for the accurate assessment of new drugs or delivery vehicles. With the increasing appearance of generic topical corticosteroid formulations which compete with trade-name equivalents, the vital importance of this assay in regulatory affairs and assessing bioequivalence has been re-emphasized. It is stressed that if the blanching assay is to be used in this sphere, then multiple-reading trials must be conducted; important registration or clinical decisions cannot be made with any validity from short-term assessments. (*J Dermatol Treat* (1991) **2**: 69–72)

Introduction

The human skin blanching assay has been used for nearly three decades as a qualitative indicator of topical corticosteroid availability and potency. This bioassay uses the skin-whitening side-effect, which follows cutaneous application of corticosteroids, to estimate the rate and extent of drug diffusion to the dermal–epidermal site of action. The extensive use of this bioassay to compare drug release from topical delivery systems has demonstrated numerous instances where drug availability varies greatly depending on the character of the delivery vehicle. It has become evident that incorporating identical concentrations of the same drug into two different topical vehicles (chemical equivalency) does not necessarily produce dosage forms that will deliver the active drug to the biosystem at the same rate or to the same extent.

It has been demonstrated in every sphere of biopharmaceutics (including the formulation of topical corticosteroids) that the substitution of one formulation ingredient for another may alter the clinical performance of the dosage form – increasing, decreasing or negligibly altering drug availability. There are several reports in the literature which illustrate the variable degrees to which the same drug is absorbed topically from different delivery formulations: fluocinolone acetonide formulations,[1] betamethasone 17-valerate formulations (Figure 1),[2,3] triamcinolone acetonide formulations[4] and halometasone products.[5] In contrast, no significant differences between blanching responses have been observed in some studies comparing proprietary products containing the same corticosteroid: diflucortolone valerate formulations,[6,7] fluocinolone acetonide formulations,[1] betamethasone 17-valerate formulations,[3,8] triamcinolone acetonide and beclomethasone dipropionate products.[7] These observations have consolidated the need for a reliable, reproducible and accurate method for comparing topical drug availability. Only in this manner can the clinical efficacies of proposed new products be accurately compared to those of existing formulations for regulatory affairs and appropriate clinical selection.

Since the initial experiments of McKenzie and Stoughton,[9] researchers have adopted numerous experimental protocols for assessing and comparing topical corticoid availability.[10,11] The skin blanching assay has been used to compare drug release from ointments,[12,13] creams and gels,[13,14] and for the comparison of generic formulations to

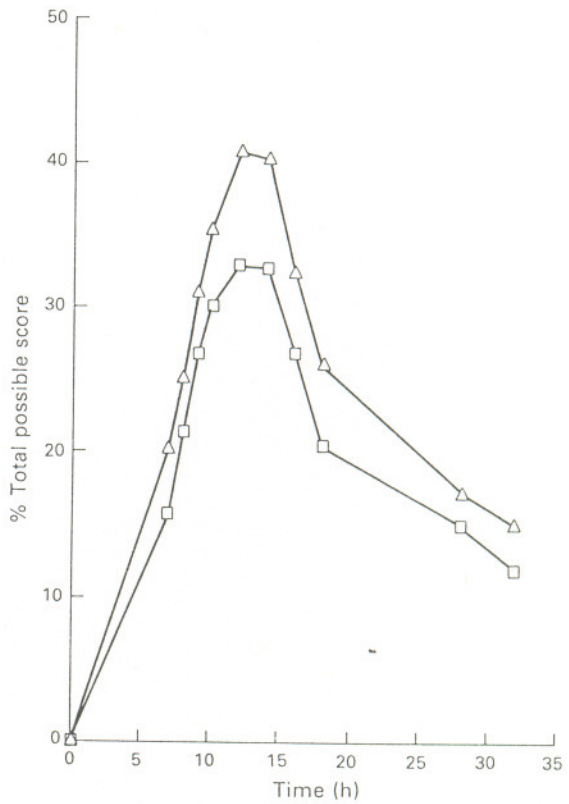

Figure 1 In vivo blanching response to 0.12% betamethasone 17-valerate contained in two different cream formulations.[2] △, Celestoderm-V; □ Betnovate.

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trade-name 'equivalents'.[15] With this multitude of experimental methodologies, problems arise in attempting to compare results from different investigators and in assessing the validity of conclusions drawn from certain experimental protocols.

Haigh and Kanfer[16] have published a detailed blanching trial procedure which attempts to minimize many of the variables and permutations common to the methodologies of other researchers. Smith et al[17] have updated this report and have specifically addressed issues such as the recording of a single versus multiple observations, the optimal contact time of the drug with the skin, and the effects of occlusive and non-occlusive dosage form application. This optimized experimental methodology has been demonstrated to be a sensitive, accurate and reproducible technique for comparing the bioavailability and potency of topical corticosteroids.[16,18]

Blanching trial methodology

The assay procedure reported by Haigh and Kanfer[16] employs 10–12 healthy, Caucasian men and women who have been pre-screened for a positive blanching response and who have not received corticoids for at least 6 weeks prior to the study. Blanching is difficult to discern on highly pigmented skin and these subjects are usually excluded from the volunteer pool. Adhesive labels, from which two 7×7 mm squares have been punched, are applied to the flexor aspects of both forearms of each volunteer. Usually, 12 discrete application sites are demarcated along the length of each forearm in this fashion. Uniform amounts of the 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 liquid preparations. The extruded formulations are spread evenly over the application sites using glass rods. Typically, 4 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, which may occur if the same pattern was used for each volunteer. The preparations are coded prior to application, maintaining the double-blind nature of the investigation. One arm of each volunteer has the preparations on the application sites occluded with strips of impervious tape, thereby preventing evaporation of moisture and delivery vehicle components. The sites on the other arm remain unoccluded, but are covered with a porous guard which will prevent accidental removal of the applied formulations by abrasion, but will not prevent exchange of moisture with the atmosphere.

The formulations remain in contact with the skin for 6 hours, after which time the guards, occlusive strips and demarcating labels are carefully removed. Residual formulation is gently washed from the application sites and the skin patted dry. The slight erythema that results from adhesive tape removal usually subsides within 30 minutes. Thereafter, 3 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 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 representing the perceived intensity of blanching at each site (0 representing normal skin, 4 representing intense blanching with distinct edges). The independent observations from the 3 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), the number of independent observers (usually 3), the number of sites per preparation per 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 AUC value by standard trapezoidal summation. A 'topical availability' 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 analyses may be applied to the results to examine the significance of the differences between profiles.

Other methods of recording the degree of blanching include a simple yes/no observation of pallor at each site, or a direct greater-than or less-than comparison of blanching at adjacent sites.[19] However these methods are generally considered to be less informative than the full curve analysis from which precise and reproducible comparisons may be made of the drug release from two preparations and of the relative potencies of the preparations if two different corticosteroids are compared. Usually drug release from a test formulation is compared to that from a standard or approved product, and often a 'reference' formulation is incorporated into the 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.[20–28] Furthermore, Engel et al[29] have noted that assessment by the blanching assay is sufficiently accurate to estimate the structure–activity relationships of topical corticosteroids, and is preferable to some other techniques. Hence, the human blanching assay appears to be a valuable tool, not only in comparing the topical availability of a corticosteroid from different delivery systems, but also in estimating the clinical usefulness of a dosage form.

Discussion

The number of blanching observations required for a reliable prediction of drug availability has been a keenly debated topic. 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.[30–32] The biopharmaceutically-accepted protocol for comparing drug bioavailability[33] suggests that such a full curve analysis would be the optimal methodology to use. Haigh et al[31] 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), one product may produce a sharp blanching profile which decreases within hours while the other may produce a relatively blunt profile that persists for a longer period. Clearly the blanching activities (and, by inference, the relative drug availabilities) are different for these products, however a single-reading blanching assay may not demonstrate this difference.

Although single observations of blanching may be useful and appropriate in specific instances for rapid screening of products,[28] 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 availability may be adequately compared by taking relatively few,[19] or even single[15,27] observations of the blanching response. Stoughton[15] has reported the results of comparing the blanching response of trade-name and generic topical corticosteroid formulations in which a single assessment of the intensity of blanching was made 18 h after initial formulation application. The 14 h contact time of formulation and skin in these experiments deviates markedly from the 6 h contact practised by other researchers, and the 18 h datum would normally fall into the post-peak region of the blanching curve described by Haigh and Kanfer.[16] Nonetheless, Stoughton suggests from these results that certain formulations may be expected to perform less satisfactorily than others when used in clinical practice. We believe that such assumptions cannot be made without analysis of the full blanching profile following multiple readings taken over a prolonged observation interval.[17,34]

Therefore, the recording of multiple readings over a prolonged time is presumed to be a pre-requisite for adequate topical availability 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 values obtained for each formulation may be important in comparing drug availability. For example, fluocinolone acetonide in topical vehicles generally elicits a slower onset of action and a prolonged blanching response (Figure 2) in comparison to other corticosteroids of similar potency.[12–14,35] This phenomenon is manifest by blanching profiles having longer t_{max} values but not necessarily lower maximum response values; also the AUC values for all the formulations compared may be equivalent. This 'sustained' performance would only be evident from multiple-reading blanching assay data. Moreover, early single-reading investigations may indicate that the fluocinolone acetonide formulation is inferior to comparative vehicles, whereas late single-readings may suggest the superiority of the fluocinolone acetonide product. Erroneous conclusions may, therefore, be drawn from this type of partial data analysis.

Furthermore, although two vehicles may differ markedly in the rate and extent to which they deliver the corticosteroid to the skin, both these rates may produce drug concentrations in the dermis/epidermis that will achieve maximal therapeutic action in clinical use. In such a case, the use of the skin blanching assay, although clearly demonstrating the different rates of drug absorption from the two vehicles, will give no indication of the equivalent therapeutic efficacy of the two formulations. Formulations classified as non-equivalent by blanching assay results may not necessarily demonstrate non-equivalent therapeutic potential, especially when inherently potent drug substances are considered.

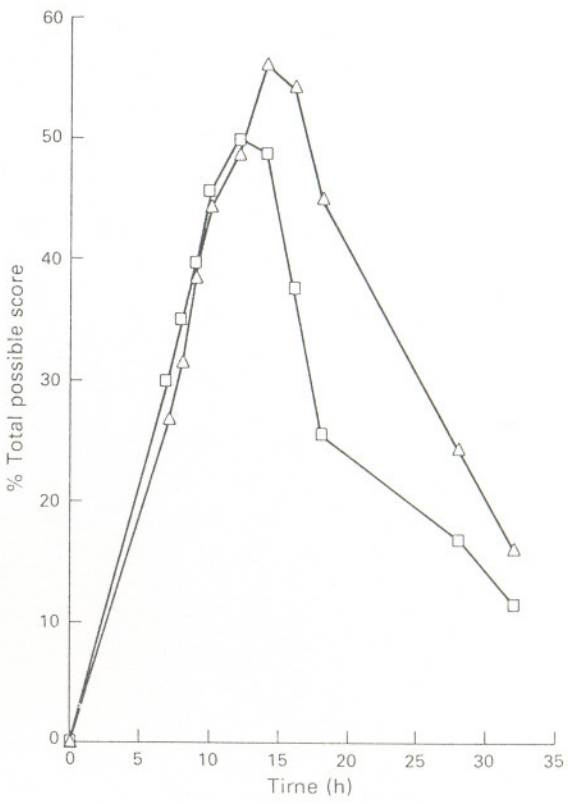

Figure 2 Blanching profiles of commercially-available 0.025% fluocinolone acetonide-containing ointment (Synalar, △) and 0.12% betamethasone 17-valerate ointment (Betnovate, □) demonstrating the slower onset and prolonged action of the former.[2]

Despite its relative crudeness when compared to modern instrumental analytical techniques, the human skin blanching assay remains a rapid and reliable comparative test of in vivo transdermal corticosteroid absorption. Moreover, it has been proved that visual and instrumental readings of the intensity of blanching yield identical results,[36] verifying the visual methodology described above. The blanching assay is attractive in that it is non-inflammatory and non-invasive, thereby subjecting the volunteers to minimal discomfort, but it is precise and accurate in predicting corticoid availability and potency. It is anticipated that the human blanching assay will remain an invaluable tool, but it should be stressed that if the bioassay is to be used for regulatory, ranking or comparative purposes then it is imperative that the full blanching curve analysis technique described[16,17] be adopted. Product registration or important clinical dermatotherapeutic decisions cannot be made on the basis of single-reading point analyses.

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