Evaluation of the Proposed FDA Pilot Dose-Response Methodology for Topical Corticosteroid Bioequivalence Testing

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Purpose. The American FDA has recently released a Guidance document for topical corticosteroid bioequivalence testing. The purpose of this study was to evaluate the recommendations of this document for appropriateness. The new specifications require a dose-vasoconstriction response estimation by the use of a Minolta chromameter in a preliminary pilot study to determine the parameters for use in a pivotal bioequivalence study.

Methods. The visually-assessed human skin blanching assay methodology routinely practiced in our laboratories was modified to comply with the requirements of the pilot study so that visual and chromameter data could be compared. Two different cream formulations, each containing 0.12% betamethasone 17-valerate, were used for this comparison.

Results. Visual data showed the expected rank order of AUC values for most dose durations whereas the chromameter data did not show similar results. The expected rank order of AUC values for both chromameter and visual data was not observed at very short dose durations. In fitting the data to pharmacodynamic models, equivalent goodness of fit criteria were obtained when several different parameter estimates were used in the model definition, however the visual data were best described by the sigmoid $E_{max}$ model while the chromameter data were best described by the simple $E_{max}$ model.

Conclusions. The $E_{max}$ values predicted by the models were close to the observed values for both data sets and, in addition, excellent correlation between the AUC values and the maximum blanching response ($R_{max}$) ($r > 0.95$) was noted for both methods of assessment. The chromameter $ED_{50}$ values determined in this study were approximately 2 hours for both preparations. At this dose duration the instrument would not be sensitive enough to distinguish between weak blanching responses and normal skin for bioequivalence assessment purposes.

KEY WORDS: human skin blanching assay; pilot dose-response study; betamethasone 17-valerate cream; pharmacodynamic modeling; chromameter.

INTRODUCTION

Over the past three decades, topical availability and potencies of corticosteroid formulations have been assessed visually using the human skin blanching assay (1). Despite its widespread use, researchers have adopted different experimental protocols to assess topical corticosteroid availability (2,3). Haigh and Kanfer (3) and Smith et al. (4) have refined the methodology so that the technique is reliable and reproducible for the assessment of topical corticosteroid formulations, provided multiple, trained observers are utilized. This, however, is considered to be too subjective by other workers (5–9). The American Food and Drug Administration (FDA) has recently released a Guidance document (6) which attempts to standardize the technique so that any assessment of bioequivalence of topical corticosteroids will be precise and accurate if the specified methodology is strictly adhered to. The Guidance as it presently stands comprises two distinct sections. Firstly, a pilot dose-response study is required, based on a dose duration method which is conducted solely with a reference listed drug. Although the dose of formulation to be applied topically is not specified in the Guidance, the objective of this pilot study is to provide the dose-response information required to determine the parameters $ED_{50}$, $D_1$ and $D_2$ to be used in the subsequent pivotal bioequivalence study. $ED_{50}$ is the dose duration equal to approximately half-maximal response and $D_1$ and $D_2$ values are dose durations which correspond to approximately 33% and 67%, respectively, of the maximal response. It has been suggested (6, Section II, p3) that this is the sensitive portion of the dose duration response curve even though there is no published evidence to support the superiority of the $ED_{50}$ value when used in topical corticosteroid bioequivalence assessments. It is interesting to note that the FDA proposes the use of this parameter which has yet to be proven of relevance to the pharmacological effect being measured in this bioassay. Secondly, a pivotal study is required to compare the in vivo response of the test product with a reference product using a dose duration which is approximately the same as the $ED_{50}$ value determined from the pilot study. The manner in which the pilot study is performed and analyzed is critical since the protocol for the pivotal study depends entirely on the results of the pilot study.

The main objectives of this study were to evaluate the pilot dose-response methodology as recommended in the Guidance in terms of the following: (a) the comparison of visual and chromameter data, (b) the comparison of blanching responses at shorter and longer dose durations and (c) the suitability of using pharmacodynamic $E_{max}$ models to describe skin blanching data. The visual assessment method, in particular, has been an area of debate in recent years (5–10), and has been deemed unacceptable (5,9) for grading the corticosteroid-induced skin blanching response, despite direct correlation between visual and instrumental data (11), and between visual data and clinical efficacy (4). It has been reported (5,10) that the use of the chromameter provides an objective and quantitative method for evaluating the intensity of skin blanching induced by topical corticosteroids. There are few published reports describing the effect of dose duration on the blanching activity of the same corticosteroid (12,13). Therefore, a comparison of visual and chromameter data, as well as the effect of various dose durations on the blanching response, are necessary for evaluation of the Guidance. The use of pharmacodynamic models for corticosteroid bioequivalence testing, as suggested in the Guidance, is a new concept with regard to the human skin blanching assay. Since there is no specific recommendation in the Guidance concerning choice of model, the selection and use of a particular pharmacodynamic fitting procedure requires investigation.

MATERIALS

The two cream formulations chosen for comparison were Betnovate (Glaxo, South Africa) and Lenovate (Lennon, South Africa)
Africavia) each containing 0.12% betamethasone 17-valerate. These two products were chosen because they have been used repeatedly as standard formulations in our laboratory and a database of results exists for these creams (1,4,14). A Minolta CR-200b Chromameter (Minolta Corporation, Ramsey, N.J.) was used. The instrument objectively records colour in terms of hue, light value and saturation. These parameters are described as the L-scale, which expresses the relative brightness of colour ranging from black to white; the a-scale, which is the colour hue related to redness or greenness and the b-scale, which is the colour range from blue to yellow. The colour of any surface can be quantitated by a combination of the three values. Theoretically, a change in these indices should reflect a change in skin colour.

METHODS

The methodology of the visual human skin blanching assay routinely practiced in our laboratories (15) was modified to comply with the specifications of the pilot dose-response study. The Guidance stipulates that there should be only one site per person for each dose duration (6, Section IV, p11). Twelve healthy male and female Caucasian volunteers with normal forearm skin and who had been pre-screened for positive blanching response in accordance with the Guidance requirements were selected. Ethical approval was obtained from the Rhodes University Ethical Standards Committee in compliance with the Declaration of Helsinki (1964) and its subsequent amendments. All subjects had previously taken part in similar studies and written informed consent was obtained from each subject. All volunteers were processed on the same day, at intervals of approximately five minutes, in order to minimize any possible effects of environmental variables such as temperature and humidity.

Five adhesive labels, from which two 7 mm × 7 mm squares had been punched, were applied to the flexor aspect of both forearms to demarcate a total of 10 application sites per arm of each volunteer. Four strips (7 mm) of each formulation (equivalent to approximately 3.2 mg) were applied in a double-blind randomized manner to each designated site and were spread using a glass rod. This is the dose of formulation normally used in this laboratory for bioequivalence testing. The formulations were applied at different times (staggered application) but removed simultaneously (synchronized removal) thus remaining in contact with the application sites for 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 5 and 6 hours. Both arms of each volunteer were left unoccluded but were protected by porous Perspex frames. After the specified contact times, the protective covers and adhesive labels were removed and the application sites were then gently washed with soap and distilled water using cotton-tipped buds and patted dry (6, Section IV, p12).

Visual Assessment of Blanching Response

Response assessment was made independently by three experienced observers at 0, 1, 2, 3, 4, 5, 6, 10, 11, 12, 19, 22, 24 and 26 hours after product removal for all dose durations. These observation periods equate to 6, 7, 8, 9, 10, 11, 12, 16, 17, 18, 25, 28, 30, and 32 hours after product application for the 6-hour dose duration. Table I lists the observation periods for the other dose durations. Responses were graded using a 0 to 4 scale where 0 = no blanching, 1 = slight blanching, 2 = more intense blanching, 3 = general even and distinct blanching and 4 = marked and very intense blanching. Visual scoring does not require separate untreated control site correction since the assessment comprises a comparison of the treated site with the surrounding unmedicated skin. The percentage of the total possible score (%TPS) was calculated (15) and plotted against time in hours after product application to produce blanching profiles for both formulations.

Chromometer Assessment of Blanching Response

The instrument was calibrated using the white calibration plate (CD-A43) immediately before the study. Blanching responses at all application sites were assessed using the a-scale parameter at the time of product removal and thereafter at intervals corresponding to the visual observations (Table I). In addition, readings were also taken at three untreated control sites on the forearm at each reading time to correct any diurnal colour change that may occur on the skin unrelated to drug exposure. The average of these readings was subtracted from the reading taken at each drug application site to yield a control site-corrected value. Zero-time chromometer values were not recorded prior to drug application since it has been shown (5,16) that no significant differences in diurnal skin colour are observed between anatomical locations on the same arm or between left and right forearms. Inclusion of the zero-time value is, therefore, a redundant arithmetical manipulation which does not impact on the final result. This was an intentional deviation from the Guidance since, theoretically, one could argue the rationale for subtracting any correction values from the chromometer readings of medicated sites, as one is attempting to obtain an absolute value of the skin colour at each observation time and monitor the change in this colour as the skin blanching progresses. The mean control site-corrected values were plotted versus time after application to conform with normal bioequivalence data reporting, since plotting procedures are not stated in the Guidance.

Statistical Analysis of Data

The trapezoidal rule was used to calculate the area under the blanching curve (AUC) for each dose duration for the visual and chromometer data. Chi-squared analyses were performed on the visual data and student’s t-distribution tests were per-