

PRECISION OF TRISTIMULUS CHROMAMETER RESULTS FROM CORTICOSTEROID-INDUCED SKIN BLANCHING

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Introduction

The human skin blanching (vasoconstriction) assay has been in use for 3 decades as a tool for the assessment of the release of corticosteroids from topical dosage forms [1]. Application of corticosteroids produces a whitening (blanching) of the skin, the intensity of which is directly related to the clinical efficacy of the formulation [1]. Assessment of the intensity of the induced blanching has classically been, and continues to be, performed by visual grading, a method which has been criticised [2] because of the subjective nature of the assessment.

Recently there has been considerable discussion in the literature [3] regarding the use of the chromameter as an objective instrumental method of monitoring corticosteroid induced skin blanching for bioequivalence assessment purposes. The FDA has released a Guidance document [4] recommending the use of the chromameter for this purpose. The chromameter measures colour in terms of three indices: the L-scale (light-dark), the a-scale (red-green) and the b-scale (yellow-blue). Any colour can be expressed absolutely in terms of these three values. The Guidance protocol suggests the use of only the a-scale values in quantifying the blanching response after correction of the data which includes subtraction of baseline and unmedicated site values. One of the unresolved issues in the FDA Guidance document is this method of data manipulation suggested since the instrument should be capable of assigning an absolute colour value to each site during the vasoconstriction period. The purpose of this study was to manipulate the instrumental data from a typical blanching study in a number of ways to investigate the appropriateness of these suggested procedures.

Materials and Methods

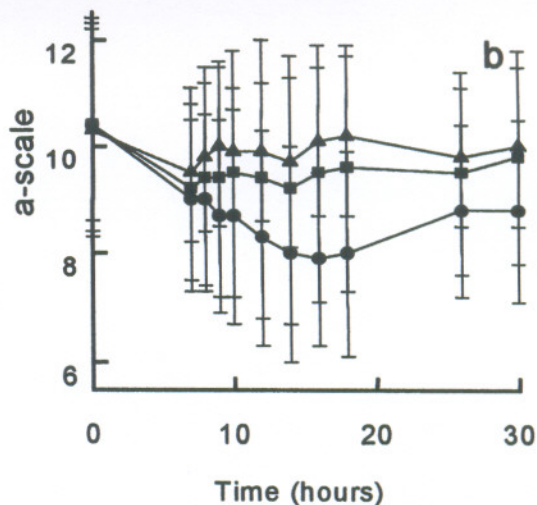
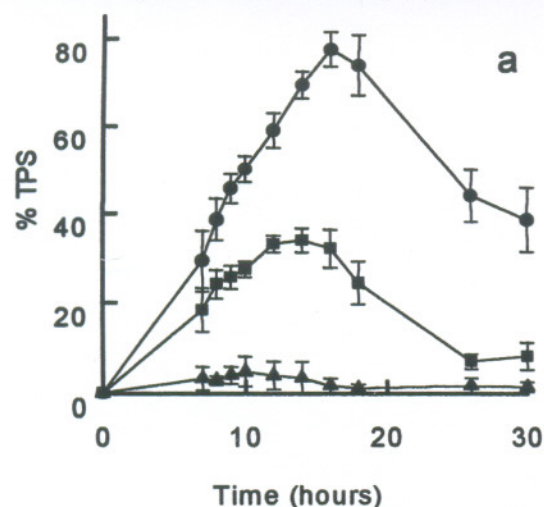
Twelve application sites were demarcated on both flexor aspects of the forearms of six, male, Caucasian volunteers. All volunteers were processed on the same day, at intervals of approximately five minutes; in order to minimise any possible effects of environmental variables such as temperature and humidity. Dovate cream (clobetasol propionate 0.05%, Pharmicare Lennon, South Africa) and Betnovate cream (betamethasone 17-valerate 0.1%, Glaxo-

Wellcome, South Africa) were each applied to four sites on each arm of each subject at a dose of approximately 3mg.cm^{-1} . The remaining four sites on each arm were left unmedicated as controls. The betamethasone 17-valerate-containing cream was selected as a standard formulation since it has been tested repeatedly in our laboratory. The trial was performed in a double-blind fashion and four different, random application patterns were utilised to prevent the appearance of a recognisable response sequence. All sites were unoccluded but were protected from accidental abrasion of the applied formulations with a plastic guard. The formulations were allowed to remain on the skin for six hours after which time they were removed by gentle washing. Blanching was monitored at 7, 8, 9, 10, 12, 14, 16, 18, 26 and 30 hours after application. Visual determinations were performed by four independent, experienced observers using standardised lighting conditions. The visual results were processed to yield blanching response profiles (%TPS) *versus* time after application. Instrumental a-, b- and L-scale readings were obtained using a Minolta CR-200 chromameter (Minolta Corporation, Ramsey, NJ, USA) which was calibrated with a standard white tile (CD-A223) before use. This allowed profiles of instrumental data *versus* time to be constructed. The uncorrected chromameter-generated data was compared to the visual data and to instrumental data manipulated by subtraction of baseline and unmedicated site values (as recommended in the FDA Guidance).

Results

Figure 1 depicts the visually-assessed skin blanching results and the uncorrected a-scale values recorded by the chromameter. The results of the visual determination of blanching show clear differences between the formulations with small standard deviations about the mean values and negligible blanching recorded for the untreated sites. This corroborates results from several previous studies performed in our laboratories. It is obvious from Figure 1 that the visual method of assessment clearly and statistically (student t-test) differentiates between the two formulations of different potency. In addition, there is clear differentiation between both formulations and the unmedicated sites. In contrast, the

Figure 1. Visual (a) and uncorrected chromameter (b) a-scale blanching profiles for Dovate (●), Betnovate (■) and Unmedicated (▲) sites.



chromameter data are remarkably imprecise; there are excessively large standard deviation bars about all the mean points with no differentiation between the means, even though there appears to be a rank order trend that mirrors the visual data. The curve for Dovate shows a similar shape to that of the visual results. The chromameter results for Betnovate do not follow the expected trend which should progress to a maximum and then regress. This trend is apparent in the visual results but not obvious with the instrumental data. These results are consistent with the data recorded in a previous Guidance evaluation study performed in our laboratory

Figure 2. Baseline- and unmedicated-corrected chromameter a-scale blanching profiles for Dovate (●) and Betnovate (■).

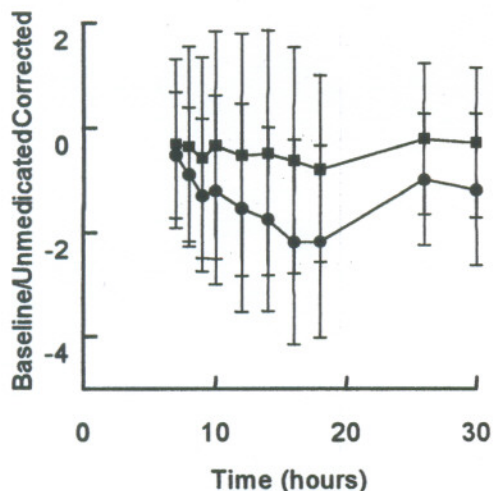


Figure 2 shows the baseline and unmedicated corrected a-scale values. Only the arithmetically-corrected a-scale data is reported here in full as this is the only one of the three chromameter scales that the FDA advocates usage of in bioequivalence studies. The corrected data plots for the L- and b-scale values are similar to those reported here for

the a-scale values. It is obvious from these figures that the correction method utilised here does not substantially change the shape of the curves or the ratios of areas under the curves obtained for the two formulations.

Discussion

In comparison to the visual data, the chromameter data is extremely imprecise and it is clear that mathematical correction of this data does not improve its quality. Furthermore, the chromameter a-index does not adequately characterise the blanching response profile. In this regard, it has been suggested [5] that Euclidean distance measurement may be a better metric on which to base an analysis of bioequivalence than the single data set methodology currently suggested by the FDA

References

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