Analysis of Chromameter Results Obtained from Corticosteroid-Induced Skin Blanching. I: Manipulation of Data

Eric W. Smith[1,2], John M. Haigh, and Roderick B. Walker

Received September 15, 1997; accepted November 12, 1997

Purpose. One of the unresolved issues in the FDA Guidance document for topical corticosteroid bioequivalence testing is the method of manipulation suggested for the chromameter data. 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 procedures for comparison with the subjective visually-assessed results.

Methods. The human skin blanching assay methodology routinely practiced in our laboratories was utilised and the vasoconstriction produced by two corticosteroid formulations of different potency was assessed visually and instrumentally by use of a Minolta chromameter. The instrumental data were corrected for zero-time and unmedicated site readings. In addition, Euclidean distances were calculated using all data generated by the instrument.

Results. Individually the a-, b- and L-scale chromameter values are imprecise and there is negligible vasoconstriction response recorded for the moderately potent formulation. Arithmetical manipulation of the data as suggested by the FDA does not appear to improve the quality of the data in any way. Euclidean distance analysis more closely resembles the visual data and appears to have better precision.

Conclusions. It is clear that mathematical correction of chromameter data is unnecessary, especially since the instrumental data are extremely imprecise. Furthermore, the assessment of each individual chromameter index does not adequately characterise the blanching response profile. It is therefore suggested that Euclidean distance may be a better measure on which to base an analysis of bioequivalence than the truncated data set methodology currently suggested by the FDA.

KEY WORDS: human skin blanching assay; chromameter; data correction; Euclidean distance; corticosteroid; skin colour measurement.

INTRODUCTION

Corticosteroid-induced skin blanching has been subjectively assessed by visual scoring since the first description of the assay procedure (1). The methodology of the assay has been adapted and optimised by several research groups (2–6) to the extent that it is accurate and reproducible provided that it is conducted by trained personnel. The visual assessment of skin blanching has been criticised (7) in that it is a technique that requires considerable training of observers and, hence, is not a robust methodology for general laboratory adoption. Clearly, an accurate, precise, reproducible and objective method for assessing the skin blanching response would be better than the subjective visual method for inter-laboratory comparison of results. Several reports have been published in the literature describing attempts to utilise diverse instrumental techniques for this purpose (8,10). None of these techniques have shown any significant advantage over visual assessment methodology and have not been adopted for routine analysis.

Recently there has been discussion in the literature (3,4,9,10,11) regarding the use of the Minolta chromameter as an objective instrumental method for monitoring corticosteroid-induced skin blanching to replace the subjective visual method. This instrument measures surface colour in terms of three indices: the a-scale (red-green), the b-scale (yellow-blue) and the L-scale (light-dark). Any homogeneous colour can be expressed absolutely by the chromameter in terms of this tristimulus analysis of the reflected xenon source light and the result may be expressed as a 3-dimensional co-ordinate. The precision obtained when assessing surfaces of non-uniform topography or colour, such as human skin, has not been extensively studied. In spite of a lack of extensive validation of this instrument, the Food and Drug Administration (FDA) has released a Guidance document (12) recommending the use of the chromameter for bioequivalence assessment purposes. The Guidance protocol suggests the use of only the a-scale values in quantifying the blanching response for this purpose, even though three separate indices are recorded for each measurement. This practice would suggest that valuable data required for the exact definition of the colour of the skin may be lost in the subsequent analysis of bioequivalence. A recent evaluation (13) of the FDA-proposed methodology using only the a-scale parameter revealed a number of weaknesses in the proposed protocol. In particular, the Guidance suggests that the chromameter-generated data be subjected to dual arithmetic correction: subtraction of baseline and unmedicated site a-scale values. The validity of this procedure has been questioned (13). It was, therefore, decided to compare the visual data generated in a typical blanching trial with uncorrected chromameter data and with chromameter data that had been corrected in different ways so as to determine whether these methods of data handling are appropriate. The visually-assessed data was used as the standard to which the instrumental data was compared for similarity as our research group has developed extensive experience and confidence in the visual trial methodology. It has been shown (14) that replicate visual determinations of skin blanching produced by the same formulation show almost identical response profiles. Therefore, it seems reasonable to suggest that comparison of the visual data with corrected and uncorrected chromameter data should give a good indication of the appropriateness of the correctional procedures employed.

The aim of this investigation was, firstly, to compare visually-assessed data separately with uncorrected a-, b- and L-scale chromameter values. This comparison would indicate if the response monitored by each of the chromameter indices mirrors the visually-assessed response. This comparison would also provide a measure of the precision of each data set since it has been reported (15) that the precision of the chromameter data is better than that of the visual data. Secondly, the visual data generated in the trial were compared to chromameter data that had been corrected in different ways so as to determine whether arithmetic methods of data handling are appropriate.

[1] School of Pharmaceutical Sciences, Rhodes University, Grahamstown 6140 South Africa.

[2] To whom correspondence should be addressed. (e-mail: paes@giraffe.ru.ac.za)

0724-8741/98/0200-0280$15.00/0

The Guidance suggests that baseline values (collected at all demarcated sites at time zero) and unmedicated site values (monitored at all the unmedicated sites throughout the period of the assay and corrected for baseline values) should be subtracted from the medicated site values to yield the data for bioequivalence evaluation purposes. We are unsure of the validity of this procedure in bioequivalence testing, as the chromameter should be capable of assigning an absolute colour value to each site during the vasoconstriction period. Therefore, it should be possible to determine a chromameter blanching response for each subject tested, regardless of the inherent skin colour of the individual. This profile would, theoretically, be equivalent to the response monitored by the eye since visual observation readily corrects for comparative differences. Moreover, if one assumes that there should be minor differences in the baseline values (B) of all the demarcated sites (11,12,13), then arithmetically it can be seen that the subtraction of B values is redundant since:

$$(M_t - B) - (U_t - B) \equiv M_t - U_t \qquad (1)$$

where M is the chromameter value recorded for a medicated site and U is the unmedicated site value at any time (t).

Thirdly, the change in the composite response of all three chromameter values, which yields absolute colour values for each application site, was compared to the visually-assessed response profile to see if the composite analysis does not better define the progression of the blanching response than each of the separate indices. The a-, b- and L-scale values can be used to define a unique point in a three-dimensional space that represents an absolute colour value. Different colours will, therefore, occupy different positions in space and the distance between two points (the Euclidean Distance (ED)) will indicate the magnitude of the difference between the colours. The ED can be calculated using co-ordinate geometry according to the following formula:

$$ED = \sqrt{(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2} \qquad (2)$$

Theoretically, as skin blanching intensity increases the ED between the medicated site data and the unmedicated site data should increase. A plot of ED *versus* time should depict the progress of the blanching response, which may show the same trends as the visually-assessed response. A more realistic evaluation of corticosteroid-induced blanching intensity using the chromameter data should be possible using all three co-ordinates produced by the instrument.

METHODOLOGY

A trial was performed using the standardised human skin blanching assay methodology (6). Twelve application sites were demarcated on both flexor aspects of the forearms of six, consenting, male, Caucasian volunteers who had not used topical or systemic corticosteroids for six months prior to the investigation. Approval was obtained from the Rhodes University Ethical Standards Committee and the study was conducted in compliance with the Declaration of Helsinki (1964) and its subsequent amendments. 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%, Pharmacare 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. The remaining four sites on each arm were left unmedicated. The betamethasone 17-valerate-containing cream was selected as a standard formulation since it has been tested repeatedly in our laboratory and an extensive database of results exists for this formulation (5,6,14,16). 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 in the usual manner (2) to yield blanching response profiles of percentage total possible score (%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 visual data profiles were compared to the chromameter-generated data, which were manipulated in one of the following ways:

1. No correction of data.
2. Subtraction of baseline (zero time) values.
3. Subtraction of unmedicated site values (corrected for baseline readings).
4. Subtraction of baseline and unmedicated site values (corrected for baseline readings) as recommended in the FDA Guidance.

The similarity in the shape of two profiles can be determined by calculation of a discrepancy factor (*d*) as follows (17):

$$d = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \times 100\% \qquad (3)$$

where R_t is the reference curve data value and T_t is the test curve data value at time t. The smaller the calculated *d* value for any two curves compared, the more similar the curves are in shape; *d* values of 0% indicate profile superimposition and values less than 20% are presumed to be very alike in profile shape. Discrepancy profile analysis was applied to the comparison of different pairs of the corrected curves to assess the likeness in their shape.

In addition, the ED values (18) were calculated between the composite a-, b- and L-scale values at each medicated site with respect to the mean of the corresponding unmedicated site values for each preparation and each subject at each observa-

tion time. These ED values were used to construct response profiles and the area under the resultant ED *versus* time plot was calculated using the trapezoidal rule.

RESULTS

Presented in Figure 1 are the results of the visually-assessed skin blanching and the uncorrected a-, b- and L-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 was recorded for the untreated sites. This corroborates results from several previous studies performed in our laboratories (19). It is obvious from Figure 1 that the visual method of assessment, despite the subjective nature of the assay, 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 chromameter data are remarkably imprecise; there are excessively large standard deviation bars about all the mean points with no differentiation between the means. However, if one examines the mean points only, there appears to be a rank order trend that mirrors the visual data. In the case of the b- and L-scale values, the more potent steroid seems to be differentiated from the moderately potent formulation and the unmedicated sites and for the b-scale results there is no rank order distinction between the data of the less potent steroid and that of the unmedicated sites. In the case of the a-scale values, there appears to be greater differentiation between the data sets, with the curve for Dovate showing a similar shape to that of the visual results. The chromameter results for Betnovate are not consistent with theoretical expectations since the blanching phenomenon should progress to a clear apex and then regress, a trend apparent in the visual results but not observed with the instrumental data. These data, therefore, do not corroborate the results of Pershing (15) who found better precision for chromameter than for visual data, but these results are consistent with the data recorded in a previous Guidance evaluation study performed in our laboratory (13).

Figure 2 shows the baseline corrected, unmedicated corrected and baseline *and* unmedicated corrected a-scale values. The ratios (Dovate:Betnovate) of the areas under the response curves are as follows: 2.1, 3.1 and 3.2. The unmedicated site

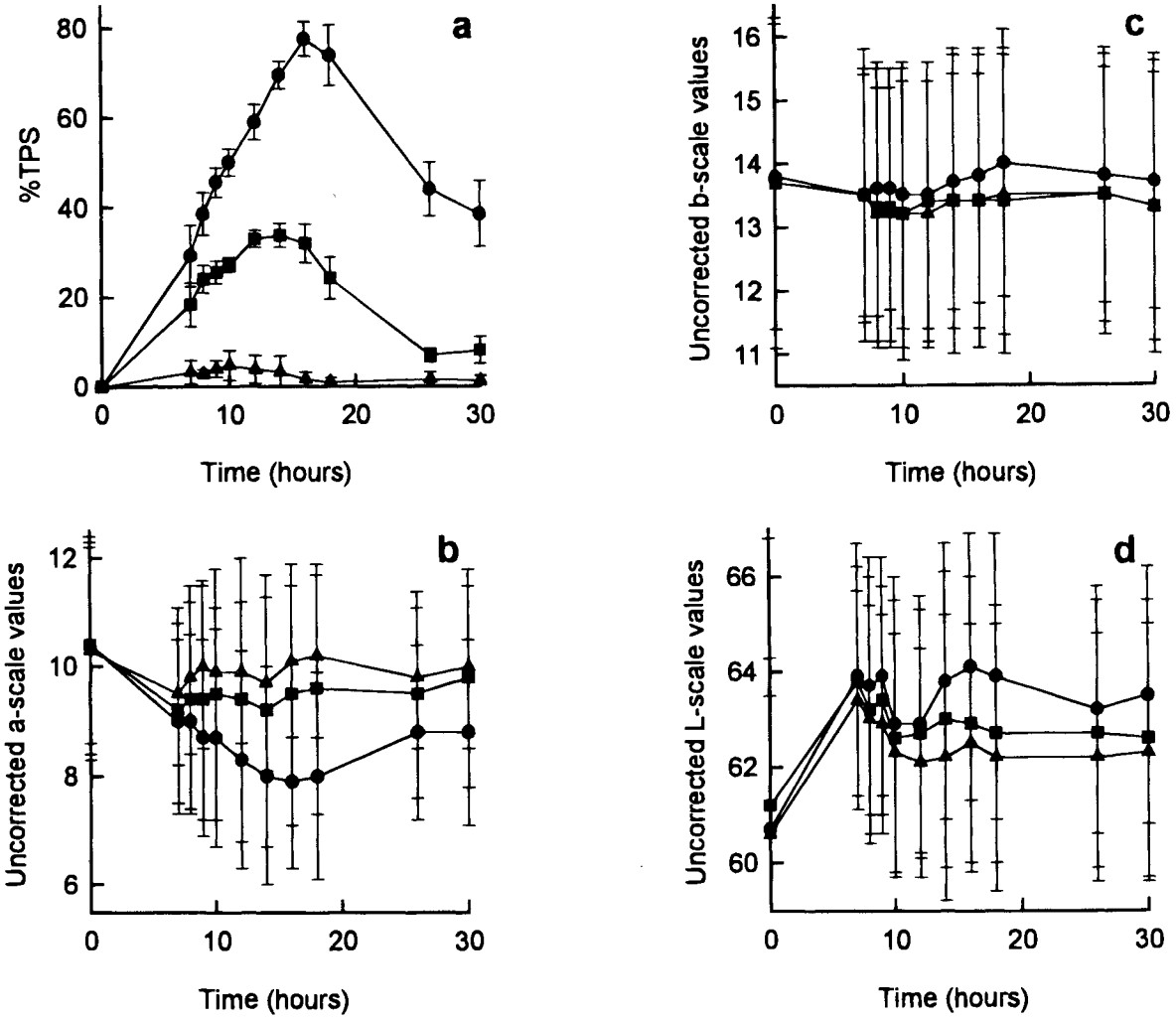

Fig. 1. Visual (a) and uncorrected chromameter (b, a-scale; c, b-scale; d, L-scale) blanching profiles for Dovate (●), Betnovate (■) and Unmedicated (▲) sites.

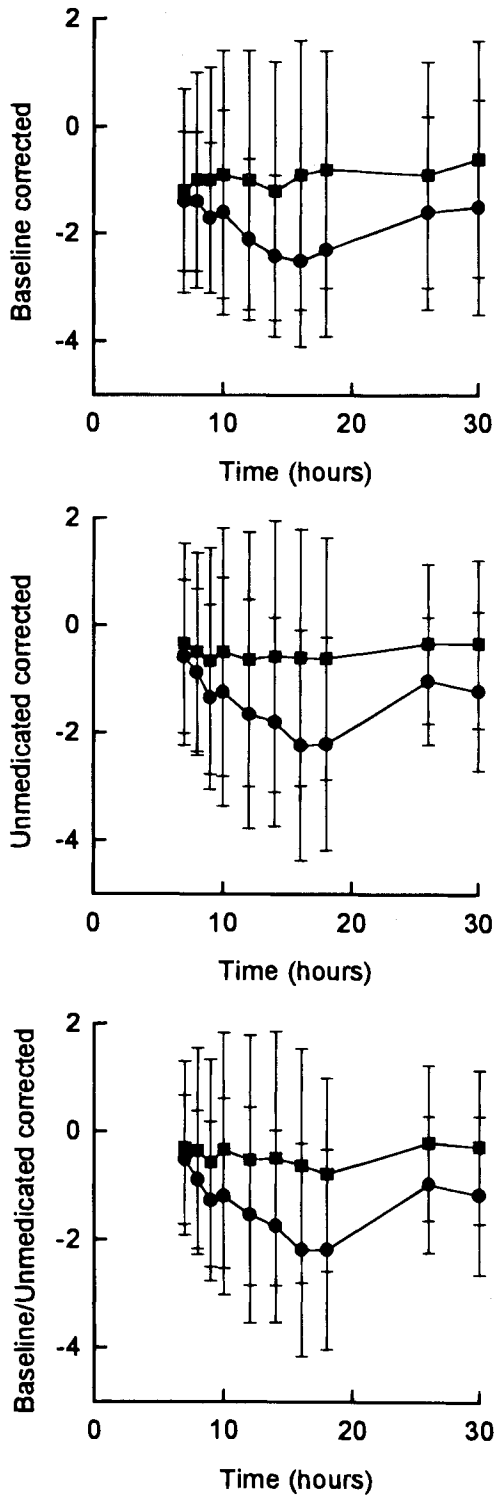

Fig. 2. Baseline-corrected, unmedicated-corrected and baseline *and* unmedicated-corrected chromameter a-scale blanching profiles for Dovate (●) and Betnovate (■).

data is not shown in these figures as these data are utilised in the three different correctional procedures. 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 quite obvious from these figures that any of the correction methods utilised here do not substantially change the shape of the curves or the ratios of areas under the curves obtained for the two formulations.

When comparing the shapes of the curves depicted in Fig. 2 using discrepancy value analysis, the following *d* values were calculated for the Dovate formulation: comparing baseline-corrected with unmedicated-corrected, 19.8%; baseline-corrected with unmedicated *and* baseline-corrected, 22.5%; and unmedicated-corrected with unmedicated *and* baseline-corrected, 3.4%. Respective values calculated for the lower potency Betnovate formulation were 43.8%, 48.6% and 13.7%. It is obvious that the curves for the higher potency formulation are much more alike than are the curves for the Betnovate formulation, clearly because of the greater imprecision of the chromameter data recorded for the latter. What is striking in this analysis is the negligible discrepancy between the data that has been corrected for unmedicated sites and the same data that has been corrected for both unmedicated sites and for baseline values (d = 3.4% for Dovate and 13.7% for the Betnovate). This further corroborates the proposition that correction for baseline values is unnecessary if one is correcting the recorded chromameter data for unmedicated site values at the same observation time (11,13).

The plots of uncorrected a-scale values *versus* data corrected in each of the three ways listed above for analysis of linearity are depicted in Figure 3. There is direct proportionality between the uncorrected and all of the corrected values for the more potent, Dovate, formulation. This trend, although present, is not as obvious for the less potent, Betnovate formulation. The linear correlation coefficient data for the Dovate plots are: uncorrected *versus* baseline-corrected (0.99), uncorrected *versus* unmedicated-corrected (0.86) and uncorrected *versus* unmedicated *and* baseline-corrected (0.85). The respective correlation coefficients for the Betnovate formulation plots are: 0.95, 0.026 and 0.002. Again, much better correlation is found for the higher potency formulation, the chromameter data for which is more precise than that of the moderately potent formulation. This corroborates earlier findings (9) that the chromameter appears to give more meaningful results when more intense vasoconstriction is measured. Clearly, if there is a linear relationship between the uncorrected data and the data that has been corrected in any of the three methods, then the need for correction must be questioned. As previously suggested (13), these results indicate that baseline correction is a redundant arithmetical manipulation. Further correction for unmedicated or baseline *and* unmedicated sites also appears to be of little merit in terms of improving the a-scale data obtained with the chromameter. Therefore, for assessment of bioequivalence, any of these data sets (including the uncorrected data) should be applicable, especially when assessed in terms of the imprecision of the raw data generated by the instrument.

In an attempt to refine the analysis of the chromameter data, the ED values were plotted *versus* time. Figure 4a represents the spatial positioning of the mean chromameter data points for all volunteers at 12 hours after formulation application. The blanching response is indicated by the Euclidean distances UB for Betnovate and UD for Dovate. The change in

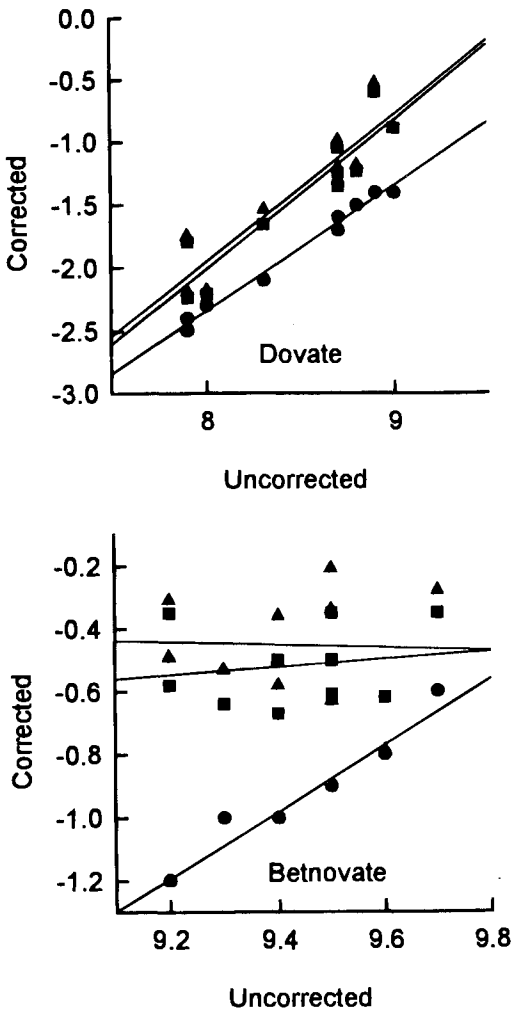

Fig. 3. Regression plots of uncorrected *versus* baseline-corrected (●), unmedicated-corrected (■) and baseline *and* unmedicated-corrected (▲) chromameter a-scale values for Dovate and Betnovate.

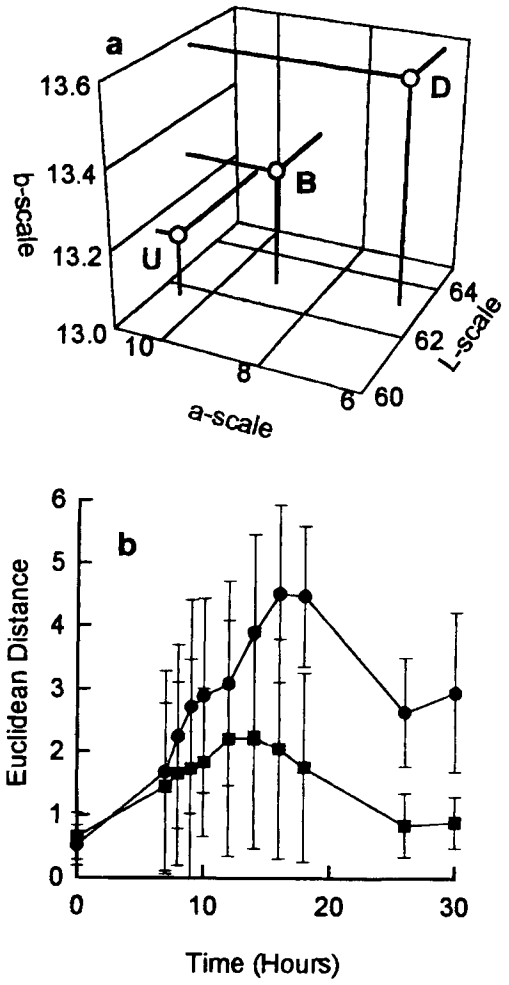

Fig. 4. a. Tristimulus Euclidean distances for Betnovate (B) and Dovate (D) formulations with respect to unmedicated sites (U) at 12 hours after formulation application. b. Euclidean distances for Betnovate (■) and Dovate (●) formulations with respect to unmedicated sites at all data observation times.

these distances throughout the trial is plotted in Figure 4b as ED *versus* time after product application. What is immediately obvious from Figure 4b is the close similarity between the shapes and relative areas of these profiles and those of the visually-assessed data. In both cases the profiles for the Betnovate formulation peak at approximately 12 hours and those of the Dovate formulation peak at approximately 15 hours. This time-to-peak trend is only mirrored by the a-scale data for the Dovate formulation. In addition, there is a clear profile defined in the ED data for the lower potency Betnovate formulation, and there are some data values at periods after peak blanching that demonstrate statistically significant differences between the responses of the two formulations. These observations are not paralleled in any of the individual a-, b- or L-value profiles that show extremely poor precision about the mean values and negligible response for the lower potency Betnovate formulation. The AUC values for the ED data were 84.7 and 42.5 for the Dovate and Betnovate curves, respectively, and the ratio of the AUC values was 2.0. In comparison, the AUC values for the visual data were 1399 and 541 for the Dovate and Betnovate curves, respectively, with a ratio of 2.6 for the two areas. It appears, therefore, that the ED treatment closely mirrors the visual data. This is not an unexpected result as colour vision has been found to be trichromatic suggesting that a single perceived colour results from the effect of three separate stimuli on the visual cortex (20).

CONCLUSIONS

Visual and chromameter data were collected in a standard human skin blanching trial in which two corticosteroid formulations of different potency were compared. The a-scale chromameter values obtained in this blanching trial were corrected for baseline site colour, for unmedicated site colour and for both baseline *and* unmedicated site colour; the latter procedure being the one suggested by the FDA. In addition, ED analysis of the three chromameter data points for each formulation at each observation time was conducted and all profiles were compared to the visually-assessed response. Individually the a-, b- and L-scale chromameter values are extremely imprecise

and there is negligible vasoconstriction response recorded for the moderately potent formulation. Arithmetical manipulation of the data as suggested by the FDA does not appear to improve the quality of the data in any way and it is, therefore, suggested that this is an unnecessary exercise since the raw data generated by the chromameter is imprecise. As the instrument, when applied in this single parameter test mode, appears incapable of monitoring a definite vasoconstriction response in the skin (which is apparent with visual observation), its applicability as specified in the Guidance for topical corticosteroid bioequivalence testing is questionable, especially for the assessment of low to moderately-potent formulations.

On the other hand, ED analysis using all three chromameter indices more closely resembles the visual data and appears to have better precision. It is therefore suggested that the use of all the data generated by the chromameter in the skin blanching assay (ED analysis) may be a better metric on which to base an analysis of bioequivalence than the truncated data set methodology currently suggested by the FDA, however, this suggested method of chromameter data manipulation requires further evaluation.

REFERENCES

1. A. W. McKenzie and R. B. Stoughton. *Arch. Derm.* **86**:608–610 (1962).
2. J. M. Haigh and I. Kanfer. *Int. J. Pharm.* **19**:245–262 (1984).
3. C. Queille-Roussel, C. Poncet, and H. Schaefer. *Br. J. Dermatol.* **124**:264–270 (1991).
4. L. K. Pershing, L. D. Lambert, V. P. Shah, and S. Y. Lam. *Int. J. Pharm.* **86**:201–210 (1992).
5. E. W. Smith, E. Meyer, J. M. Haigh, and H. I. Maibach. *J. Dermatol. Treat.* **2**:69–72 (1991).
6. E. W. Smith, E. Meyer, J. M. Haigh, and H. I. Maibach. In R. L. Bronaugh and H. I. Maibach (eds.), *Percutaneous Absorption* Vol. 2. Marcel Dekker, New York, 1989, pp. 443–460.
7. V. P. Shah, C. C. Peck, and J. P. Skelly. *Arch. Dermatol.* **125**:1558–1561 (1989).
8. E. W. Smith, E. Meyer, J. M. Haigh, and H. I. Maibach. In R. L. Bronaugh and H.I. Maibach (eds.), *Percutaneous absorption. Mechanisms—Methodology—Drug Delivery*. Vol. 2. Marcel Dekker, New York, 1989, pp. 443–460.
9. P. Clarys, L. Wets, A. Barel, and B. Gabard. *J. Eur. Acad. Dermatol. Venereol.* **5**:250–257 (1995).
10. S. Y. Chan and A. Li Wan Po. *J. Pharm. Pharmacol.* **44**:371–378 (1992).
11. M. J. Waring, L. Monger, D. A. Hollingsbee, G. P. Martin, and C Marriott. *Int. J. Pharm.* **94**:211–222 (1993).
12. Guidance: topical dermatologic corticosteroids: *in vivo* bioequivalence. Division of Bioequivalence, Office of Generic Drugs, Food and Drug Administration, Rockville, MD. June 1995.
13. P. H. Demana, E. W. Smith, R. B. Walker, J. M. Haigh, and I. Kanfer. *Pharm. Res.* **14**:303–308 (1997).
14. J. M. Haigh, E. Meyer, E. W. Smith, and I. Kanfer. *Int. J. Pharm.* **152**:179–184 (1997).
15. L. K. Pershing. *Drug Info. J.* **29**:923–934 (1995).
16. E. W. Smith, E. Meyer, and J. M. Haigh. *Drug. Res.* **40**:618–621 (1990).
17. J. W. Moore and H. H. Flanner. *Pharm. Tech.* **20**:64–74 (1996).
18. P. Clarys, A. O. Barel, and B. Gabard. In K. R. Brain, V. J. James and K. A. Walters (eds.), *Prediction of Percutaneous Penetration.* Vol. 3b. STS Publishing, Cardiff, 1993, pp. 502–510.
19. E. W. Smith, E. Meyer, and J. M. Haigh. In H. I. Maibach and C. Surber (eds.), *Topical Corticosteroids.* Karger, Basel. 1992, pp. 65–73.
20. R. S. Hunter and R. W. Harold. *The Measurement of Appearance*, 2nd Edition, Wiley-Interscience, New York, 1987.