COMPARISON OF VISUAL CR-200 AND CR-300 CHROMAMETER DATA OBTAINED FROM THE CORTICOSTEROID-INDUCED SKIN BLANCHING ASSAY

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Introduction

In a recent Guidance document the American FDA recommended the use of a chromameter rather than the human eye for the assessment of the pharmacodynamic blanching response produced after topical application of corticosteroids. The purpose of this study was to investigate the appropriateness of the human eye and two types of chromameter for the estimation of skin blanching.

Experimental Methods

Four cream formulations, each containing 0.12\% betamethasone 17-valerate were chosen for this investigation: Adco-Betamethasone (Adcock Ingram, South Africa), Betnovate (Glaxo-Wellcome, South Africa), Celestoderm-V (Schering-Plough, South Africa) and Lenovate (pharmacare-Lennon, South Africa). Two Minolta chromameters were used, the CR-200 and the CR-300 (Minolta Corp., USA) which underwent a standard inspection procedure (Minolta Ltd., Switzerland) prior to this investigation.

The methodology of the human skin blanching assay routinely practiced in our laboratories \cite{1} was modified to comply with the aims of this study. Twelve healthy male and female light-skinned volunteers (aged from 18-25) who had been pre-screened for positive blanching response were selected. Ethical approval was obtained from the Rhodes University Ethical Standards Committee in compliance with the 1964 Declaration of Helsinki and its subsequent amendments. Written informed consent was obtained from each subject.

Application of formulation

All volunteers were processed sequentially at 5-min intervals in order to minimize any possible effects of environmental variables such as temperature and humidity. Six adhesive labels, from which two 7 x 7 mm squares had been punched, were applied to the flexor aspect of both forearms to demarcate a total of 12 application sites per arm of each volunteer. Each formulation was applied to three of the demarcated sites in a random manner \cite{2} and four adjacent sites were utilized for the control. The formulations were applied by extrusion of four stripes (equivalent to approximately 3.2 mg \cite{3}) from a 1-ml syringe to each designated site in a double blind, randomized manner. The extruded formulations were spread evenly over the application sites using a glass rod, and were covered with a porous Perspex frame to prevent accidental removal of the applied formulations. After a contact time of six hours, the protective covers and adhesive labels were removed and each application site was separately washed using cotton-tipped buds and distilled water and patted dry.

Visual assessment of blanching response

Response assessments were made independently by three experienced observers at 7, 8, 9, 10, 12, 13, 14, 15, 16, 18, 26, 28 and 32 hours after product application. Standard overhead fluorescent lighting was used to illuminate the horizontally-placed arms of the volunteers. Responses were graded using a ordinal 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. The percentage of the total possible score (%TPS) was calculated \cite{1} and plotted against time in hours after product application to produce blanching profiles.

Chromameter assessment of blanching response

The instruments were calibrated using the white calibration plate (CR-A43) immediately before the study. Baseline readings (zero time) were taken at all sites (including the untreated control sites) prior to the application of the formulations. Thereafter, blanching responses at all application sites and at four untreated control sites were assessed at the same times as the visual assessments. Readings with both the CR-200 and the CR-300 chromameters (L-, a-, and b-scale parameters: only a-scale are reported here; L- and b-scale produced similar profiles) were taken in the same room by two different investigators. The arms of the volunteers were placed horizontally for these measurements.
Results

The topical bioavailabilities of the four creams were determined using visual scoring and chromameter measurements. The weakest blanching was observed for Celestoderm-V, Betnovate and Adco-Betamethasone creams which showed similar blanching responses, whereas Lenovate cream showed a superior response. Good correlation between the visual assessments made by three independent observers and moderate to good correlations between visual, CR-200 and CR-300 measurements were observed. No direct linear relationship between the AUBCs produced by the two chromameters was observed. This investigation showed that the use of the chromameter is not completely objective as has been suggested previously, since the alignment of the measuring head, the force of the application of the head to the skin and the orthostatic placement of the arms have a considerable effect on the readings obtained from this instrument.

Conclusions

Visual scoring and chromameter measurement are both valid means for the assessment of topical corticosteroid bioequivalence in the human skin blanching assay. Each procedure needs to be validated and investigators have to be trained for both visual assessment and the operation of the chromameter.

Acknowledgments

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References

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