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The human skin blanching assay for in vivo topical corticosteroid assessment I. Reproducibility of the assay

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Abstract

The human skin blanching (vasoconstriction) assay for the assessment of topical corticosteroids has been in use for over 30 years, the intensity of the drug-induced blanching being assessed subjectively by eye. Both arms of several male and female volunteers are used for product application and more than one observer is used to estimate the degree of induced blanching. There are, therefore, numerous variables which are inherent in the assay procedure. This investigation consisted of three identical trials performed at 8-week intervals, utilising the same 18 volunteers and the same three observers in an attempt to address the question of reproducibility of the assay. From the results obtained it is clear that the assay methodology is capable of consistently distinguishing, on a rank order basis, between preparations which show similar blanching (chemically-equivalent formulations). The similarity of the results for the three individual trials gives considerable confidence to results produced using this methodology. An experiment designed to test the reproducibility of the blanching scores showed that the observers are capable of producing identical results even though visual observation is highly subjective. © 1997 Elsevier Science B.V.

Keywords: Topical corticosteroids; Human skin blanching assay; Reproducibility

1. Introduction

The human skin blanching assay has been in use for over 30 years (McKenzie and Stoughton, 1962; Haigh and Kanfer, 1984; Noon et al., 1996) and has proved itself to be a reliable assay procedure for the assessment of topical corticosteroids. The basis of the assay is the measurement of the side effect of blanching (vasoconstriction) of the skin caused by topical application of glucocorticoids. The exact mechanism of the production of blanching is not fully understood, but it is thought that local vasoconstriction and the consequent blood flow reduction is the cause of this phenomenon. For many years this assay was referred to as the vasoconstrictor assay (Woodford and Barry, 1984). Since no direct measurement of

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vasoconstriction is made and since it is the degree of blanching which is assessed, we prefer to call this technique the human skin blanching assay.

The measurement of blanching is performed by use of the human eye, an obviously highly subjective method. There are other objective methods available for the measurement of blanching in human skin (Clarys et al., 1995; Montenegro et al., 1996) which will be discussed in the second paper in this series (Haigh et al., 1997). Male and female volunteers are normally included in each experiment, both arms of each individual are used as application sites and invariably more than one observer is used to estimate the degree of induced blanching. There are, therefore, a large number of variables which are inherent in the assay procedure. These reports will attempt to assess this variability and propose the best possible parameters so that this assay may be performed with confidence.

2. Materials and methods

This investigation consisted of three identical trials performed at 8-week intervals, utilising the same volunteers and the same observers. Eighteen healthy Caucasian subjects (9 male, 9 female) were selected from a panel of volunteers known to consistently demonstrate a blanching response to topically applied corticosteroid formulations. The flexor aspect of each arm of each individual was utilised; 12 application sites were demarcated by applying six self adhesive labels, from which two 7×7 mm holes had been punched, onto each forearm.

Two commercially available topical corticosteroid formulations (Betnovate cream, Glaxo, South Africa and Celestoderm-V cream, Schering-Plough, South Africa), both containing 0.12% betamethasone 17-valerate, were tested since this assay is normally utilised for bioequivalence studies on chemically-equivalent formulations. The possibility of using two formulations containing different corticosteroids was considered, but rejected as very different degrees of blanching would have been elicited, making observer decisions facile.

Both formulations were assayed by HPLC (Smith et al., 1985) immediately after applications were made for each trial and were found to contain the same concentration of betamethasone 17-valerate (Table 1). The same two tubes of formulation were used for all three trials. Each formulation was applied to six sites per arm using a disposable plastic syringe which was filled immediately prior to use in order to minimise any possible interaction between the corticosteroid and the matrix of the barrel. The needles were cut to 5 mm to facilitate extrusion of the formulation. Four 7 mm stripes ($\pm$ 3.2 mg) of the formulation were applied to each site and spread with a blunt glass rod, one for each formulation. Four different application patterns were used to prevent the appearance of a discernable pattern. The six sites occupied by any one formulation were distributed over the whole length of the forearm as it has previously been demonstrated that the degree of induced blanching varies on different areas of the forearm (Meyer et al., 1992). Both arms of each individual were left unoccluded but protected with a perspex guard to prevent accidental abrasion of the formulations. All volunteers were processed sequentially at 5 min intervals in order to minimise any possible effects of environmental variables such as temperature and relative humidity. The formulations were left in place for 6 h, after which time the labels were removed and any residual cream was washed off the forearm with soap and warm water which was then gently patted dry.

Blanching responses were assessed by three independent, experienced observers at 7, 8, 9, 10, 12, 14, 16, 18, 28 and 32 h after application under standard lighting conditions. The arms were held

Table 1
Assay concentration of betamethasone 17-valerate and AUBC values of the two formulations used in this investigation

	Celestoderm-V		Betnovate	
	Assay (%)	AUBC	Assay (%)	AUBC
Trial	0.119	783	0.120	611
Trial 2	0.122	785	0.120	691
Trial 3	0.121	724	0.121	593

horizontally on a flat surface directly in front of the observer. Blanching was assessed on a 0–4 scale where 0 represents no blanching, 4 represents intense blanching and 1, 2 and 3 represent intermediate grades.

All volunteers were housed in the same laboratory until after the 18-h reading. Applications started at 7:30 a.m. on day one and the blanching estimations commenced at 3:00 p.m. on the same day. Readings were made on volunteers in the same order in which applications had been made thus ensuring equal time differences. The 18-h readings of blanching were performed at 2:00 a.m. on day 2 when volunteers were allowed to leave the facility, return home to sleep and report at 12 noon on day 2 for the penultimate blanching estimations. The final readings were performed at 4:00 p.m. on day 2. Volunteers were required to abstain from any exercise, bathing, consumption of alcohol or use of medication and all meals were standardised.

The blanching response results were calculated as a percentage of the total possible score (percentage TPS) as previously described (Haigh and Kanfer, 1984). The percentage TPS values were used to plot the blanching profiles and the area under the blanching curve (AUBC) was calculated using the trapezoidal rule with a cut-off at the 32-h reading.

A further experiment was designed to assess the reproducibility of the observer-generated blanching profiles. Three formulations were used in this experiment, but, unbeknown to the observers, two of these were exactly the same formulation. The experimental details for this trial were the same as for the previous trial, except that three formulations were applied to the test sites as opposed to two and one arm of each volunteer was occluded with non-porous plastic tape, the other left open but protected with a perspex guard to prevent accidental abrasion of the formulations.

3. Results and discussion

The comparative blanching responses of Celestoderm-V cream and Betnovate cream were assessed for all three trials. The blanching profiles depicted in Figs. 1–3 represent the pooled results recorded by the three observers for both arms of all 18 volunteers for each trial. Since each formulation was applied to six sites per arm of 18 volunteers and blanching was estimated by three independent observers, it follows that at each reading time the blanching of any one formulation was estimated 648 times. The AUBC values (Table 1) for Celestoderm-V were almost identical for trials 1 and 2, with trial 3 being slightly lower at 92% of the values of trials 1 and 2. There was greater variation in the case of Betnovate, trial 2 producing the highest value and trial 3 giving the lowest value which was 86% of the highest. Maximum blanching was observed for both formulations at 12 h after application in all three trials. It

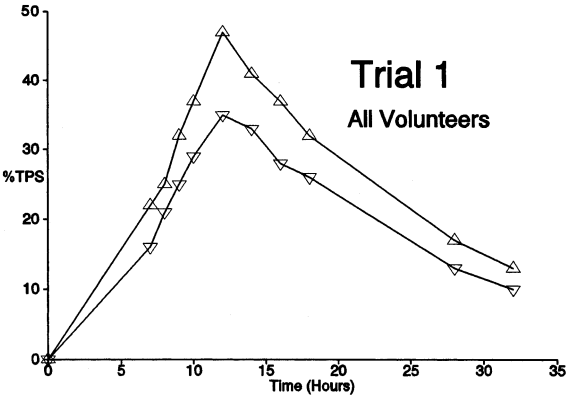

Fig. 1. Blanching profiles for both formulations, all volunteers and all observers for trial 1. △, Celestoderm-V; ▽, Betnovate.

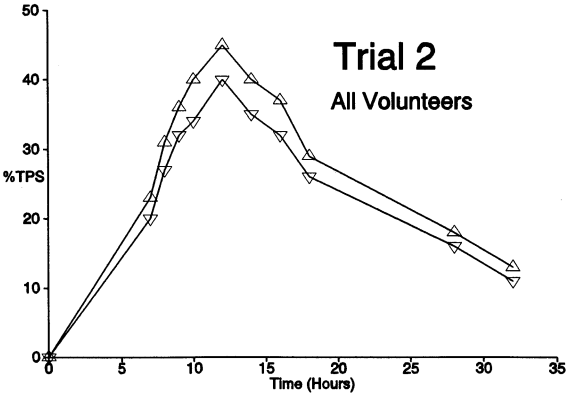

Fig. 2. Blanching profiles for both formulations, all volunteers and all observers for 2. △, Celestoderm-V; ▽, Betnovate.

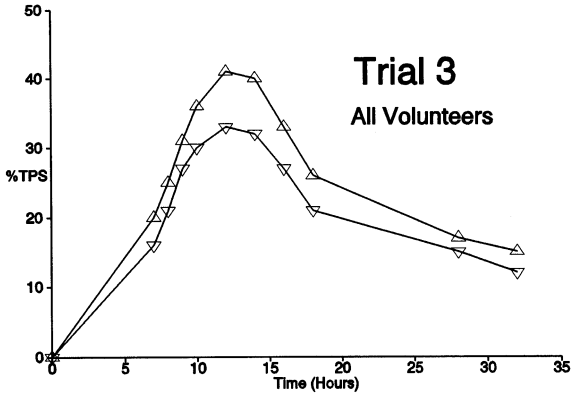

Fig. 3. Blanching profiles for both formulations, all volunteers and all observers for trial 3. △, Celestoderm-V; ▽, Betnovate.

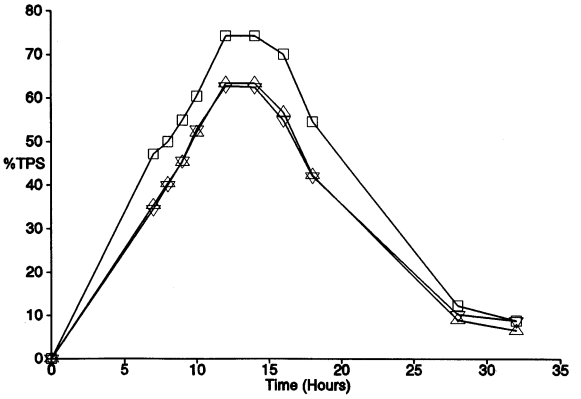

Fig. 4. Blanching profiles in the occluded mode for three formulations. □, formulation 1; △, formulation 2; ▽, formulation 3; formulations 2 and 3 are identical.

can be seen from these profiles that Celestoderm-V elicited a more intense blanching response than Betnovate in all three trials, indicating reproducibility in terms of the rank order of the formulations.

A previous attempt to show the reproducibility of the human skin blanching assay (Barry and Woodford, 1978) reported ten trials of the same formulation (Betnovate cream) determined over a period of 3.5 years. For these trials, the volunteer panel was variable as was the observer panel. The maximum %TPS values varied from under 70 to over 90%. This variability is almost certainly due to the lack of consistency between the trials.

Another retrospective study of Betnovate cream-induced skin blanching (Smith et al., 1992) utilised different subjects and different observers over a period of 11 years but showed less variability, probably due to the large number of determinations (59 670 observations).

Figs. 4 and 5 show the results of the testing of the reproducibility of the observer data. It can be seen that the preparations which were applied as different formulations, but which in fact were exactly the same, produced blanching curves which are almost identical. In the occluded mode the AUBC values varied by a single unit (1003 and 1004) and in the unoccluded mode, the difference was slightly larger (542 and 556). This marginally greater difference might have been due to the increased difficulty of assessing weaker blanching responses. Whilst it cannot be denied that the visual method of observation utilised in the human skin blanching assay is subjective, the reliability of visual assessment performed by experienced observers is certainly demonstrated by the similarity of the blanching responses reported here.

4. Conclusions

From the present study it is clear that the assay method is capable of consistently distinguishing, on a rank order basis, between chemically-equivalent formulations. Since this is the main reason

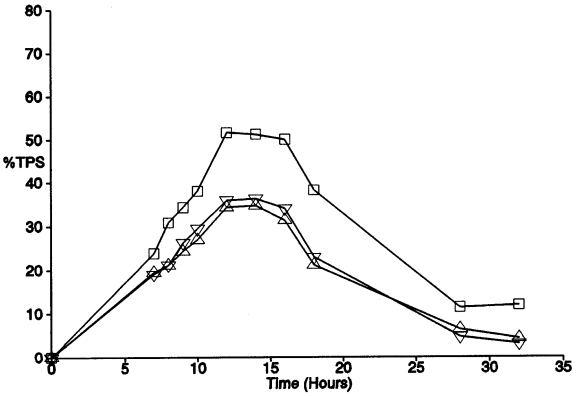

Fig. 5. Blanching profiles in the unoccluded mode for three formulations. □, = formulation 1; △, formulation 2; ▽, formulation 3; formulations 2 and 3 are identical.

for conducting assays of this type, the similarity in the results for the three individual trials gives considerable confidence to results produced from this methodology. There are, however, differences between the blanching profiles for each formulation for each of the three trials, as would be expected. The experiment designed to demonstrate the reproducibility of the blanching scores as assessed by the observers showed that they are capable of producing virtually identical results even though the method is highly subjective.

Acknowledgements

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