In Vivo/In Vitro Assessments of Topical Hydrocortisone Availability: Correlation Between Blanching Assay and Laboratory Cell Experiments

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I. INTRODUCTION

Topical corticosteroids are still the most widely used drugs in the treatment of dermatological conditions. Early corticosteroid dosage forms consisted of
simple creams or ointments where more emphasis was placed on the potency of the drug molecule than on the intrinsic delivery potential of the vehicle. More recently, the effect that the composition of the semisolid base has on the extent of drug delivery has been researched to a much greater extent. These advances in the science of dosage form design have necessitated the refinement of precise and accurate methods for testing the drug delivery efficacies of the developed products. Obviously, the best method for the assessment of the effectiveness of corticosteroid formulations is in a therapeutic situation. Clinical trials, however, are fraught with methodological problems that make duplication of a trial impossible. Alternatively, a number of pharmacological models exist for this type of assessment, but it is often problematic to obtain correlation with the true dermatological conditions.

The human skin blanching assay is one of the most reliable and reproducible of the in vivo methods available for the assessment of topical corticosteroid formulations. The skin whitening (blanching or vasoconstriction) side-effect that follows corticosteroid application was first utilized in 1962 as a measure of the percutaneous absorption of corticosteroids from topical formulations. Optimization of this initial procedure has produced a reliable and precise bioassay methodology for the assessment of the efficacy of topical corticosteroid formulations.

One criticism of this assay has been the subjective nature of the observation procedure. Although these points have repeatedly been addressed in the literature, it has been suggested that it would be beneficial to have some in vitro penetration data to supplement in vivo observations, as this would strengthen the assessment of the topical equivalence of similar delivery formulations. With this objective in mind, a comparison of hydrocortisone release from two proprietary cream formulations was compared by in vivo and in vitro techniques to determine if any correlation could be established between the methodologies.

II. EXPERIMENTAL METHODOLOGY

The two commercial cream formulations used in this comparison were Cutaderm cream (Scherag, South Africa) and an experimental cream formulation (Lennon, South Africa), each containing hydrocortisone at a concentration of 0.5%. Both formulations were assayed by a modified high-performance liquid chromatography technique and were found to contain equivalent concentrations of hydrocortisone.

A. IN VIVO METHODOLOGY

The optimized methodology of the human skin blanching assay has been reported in detail previously. Briefly, the assay used here employed the
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forearms of 12 subjects who had been screened for blanching response to topical corticosteroids. Four application patterns were used, each comprising 12 sites to which the formulations to be compared were randomly assigned so that each formulation was represented six times along the entire length of the forearm. The arm of each volunteer was randomly assigned one of the four patterns. Self-adhesive labels that had two 7 x 7-mm squares punched from their centers were used to demarcate the 12 forearm sites. Approximately 3 mg of each cream to be compared was extruded onto each site, according to the application patterns, from a small syringe, which had its needle cut to approximately 5 mm, and the formulation was uniformly spread using a glass rod. Once all the sites had been filled, the arm of each volunteer was occluded by covering each pair of sites with water-impervious tape. Only the occluded application mode was used here as this relatively weak corticosteroid, in low concentration, induces only slight blanching when tested without occlusion, which makes observation and interpretation of the results more difficult. The formulations, adhesive tapes, and dressings remained on the skin for 6 h, after which they were gently removed and any residual formulation washed from the skin. The first observation of skin blanching was made 1 h after product removal (7 h after application).

Three trained observers were used in this double-blind assessment to counteract the subjectivity of the methodology. The blanching response was independently assessed by the observers on nine occasions between 7 and 32 h after application. The intensity of blanching was graded on a 0 to 2 scale, 0 representing no blanching, 2 representing the most intense blanching expected, and 1 representing the intermediate grade. After the final observation the scores were decoded and the data from the three observers were summated for generation of a response profile (sum of the product of the frequency and observed score):

$$\text{Summated score} = \sum_{x=0}^{x=2} f(x)$$

The blanching response profiles generated in this way allow visual ranking of the formulations for comparative purposes. The qualitative data generated in this assay are statistically compared, for topical equivalence purposes, by nonparametric ($\chi^2$) techniques.

B. IN VITRO METHODOLOGY

The in vitro permeation cell methodology used in this study has been described previously. Custom-made, glass diffusion cells of 10-ml volume were utilized in the vertical, “Franz” mode to measure the rate of hydrocortisone penetration from each cream formulation (packed into the donor chamber), through 1.65-cm² area full thickness hairless mouse skin, to a receptor
phase of 10 ml purified isopropyl myristate maintained at 35°C and agitated by Teflon-coated bar stirrers at 600 rpm. The full techniques for membrane harvesting and preparation\textsuperscript{17} and for high-performance liquid chromatographic analysis of the permeant in the receptor phase\textsuperscript{18} have been reported in detail. In addition, the corticosteroid penetration performance of this cell design has been fully evaluated.\textsuperscript{16}

III. RESULTS

A. \textit{In Vivo Assessment}

The results of the human skin blanching assay are presented in Table 1 and are plotted in Figure 1 as the blanching response (summated score) versus the time after initial application of the formulations to the skin. These response profiles indicate that the blanching elicited by the experimental hydrocortisone formulation is greater in rank order than that of the Cutaderm\textsuperscript{®} formulation. However, the profiles are similarly shaped and both peak at approximately 12 h. At face value, these drug delivery profiles do not appear to be significantly different, except possibly toward the end of the observation time where there is some divergence of the plots observed.

\begin{table}[h]
\centering
\caption{In Vivo Blanching Response Data for Commercial and Experimental Hydrocortisone Cream Formulations Applied in the Occluded Mode} \label{table1}
\begin{tabular}{lcccccccccc}
\hline
\textbf{Score} & \textbf{7} & \textbf{8} & \textbf{9} & \textbf{10} & \textbf{12} & \textbf{14} & \textbf{16} & \textbf{18} & \textbf{28} \\
\hline
\textbf{Cutaderm\textsuperscript{®}} & & & & & & & & & \\
0 & 144 & 86 & 80 & 66 & 59 & 71 & 113 & 143 & 181 \\
1 & 70 & 128 & 129 & 137 & 123 & 124 & 96 & 73 & 35 \\
2 & 2 & 2 & 7 & 13 & 34 & 21 & 7 & 0 & 0 \\
\hline
\textbf{Summated score} & 74 & 132 & 143 & 163 & 191 & 166 & 110 & 73 & 35 \\
\hline
\textbf{Hydrocortisone} & & & & & & & & & \\
0 & 129 & 97 & 81 & 63 & 60 & 68 & 105 & 130 & 174 \\
1 & 84 & 113 & 124 & 127 & 113 & 108 & 96 & 76 & 40 \\
2 & 3 & 6 & 11 & 26 & 43 & 40 & 15 & 10 & 2 \\
\hline
\textbf{Summated score} & 90 & 125 & 146 & 179 & 199 & 188 & 126 & 96 & 44 \\
\hline
\end{tabular}
\end{table}
IN VIVO BLANCHING ASSAY RESULTS

FIGURE 1 Human skin blanching response versus time after application profiles for commercial and experimental hydrocortisone cream formulations. Significant differences at the 95% level denoted by ↓.

B. IN VITRO ASSESSMENT

The rate of hydrocortisone permeation through full thickness hairless mouse skin under these experimental conditions is, essentially, equivalent from both cream formulations (Table 2). As can be seen from Figure 2, the two profiles intersect at various times and should show no statistically significant differences at any sampling time. The flux profiles for both creams appear approximately linear, especially during the first 24 h, with a slight increase in the hydrocortisone permeation rate observed at later sampling times. From these data it may be concluded that there is no difference in the rate of drug release or diffusion through hairless mouse skin from Cutaderm® cream and the experimental 0.5% hydrocortisone formulation.

The sensitivity of the high-performance liquid chromatographic technique employed in this assay allows receptor chamber hydrocortisone permeation flux as low as 0.5 μg cm⁻² to be quantified. It is therefore possible to adequately monitor drug permeation with this cell system during the initial period of experimentation, the first quantifiable sample being withdrawn at 6 h. The lack
IN VITRO PERMEATION RESULTS

FORMULATIONS
- CUTADERM®
- HYDROCORTISONE

FIGURE 2  
In vitro penetration of hydrocortisone through hairless mouse skin at 35°C from a commercial and an experimental cream formulation.

TABLE 2

In Vitro Drug Permeation Data for Commercial and Experimental Hydrocortisone Cream Formulations

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Mean drug mass permeating in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cutaderm®</td>
</tr>
<tr>
<td>6</td>
<td>0.59 (0.39)a</td>
</tr>
<tr>
<td>12</td>
<td>1.52 (0.39)</td>
</tr>
<tr>
<td>24</td>
<td>3.40 (0.44)</td>
</tr>
<tr>
<td>31</td>
<td>4.94 (0.66)</td>
</tr>
<tr>
<td>48</td>
<td>8.85 (0.35)</td>
</tr>
<tr>
<td>72</td>
<td>16.92 (1.48)</td>
</tr>
</tbody>
</table>

* Mean drug mass permeating hairless mouse skin at 35°C and 600 rpm agitation (µg cm⁻²).

b Standard deviations of means in parentheses.
of an appreciable lag phase in the initial monitoring period would suggest that the permeant equilibrates rapidly with the barrier membrane and diffuses rapidly through it, into the receptor chamber. The laboratory data obtained here are also in congruity with results published for hydrocortisone penetration through synthetic membranes. The latter study recorded approximately tenfold greater drug diffusion through the synthetic media than was observed for the hairless mouse skin in this study, a difference that would be expected, bearing in mind the general permeabilities of these two sets of membranes.

C. STATISTICAL ANALYSIS

At face value, there appears to be no significant difference in the visually assessed blanching response profiles of the two formulations. The chi-squared ($\chi^2$) distribution test was employed to determine the significance of the difference between the qualitative observations of the skin blanching intensity generated by the two formulations at each reading time. A two-by-two contingency table was used at each observation time that included the frequencies of the two highest grades of blanching observed (numbers of 1 and 2 grades). For the purposes of topical equivalence estimation, the period of maximal blanching (10 to 16 h) is considered most important. These data are depicted graphically in Figure 3 as the frequencies of each grade of blanching observed for each formulation over the peak response period. Significant differences in the

![Frequencies of grades 1 and 2](image-url)
data were found by this method at the 10- and 14-h observation times, with no significance in the difference being found at 12 and 16 h. Statistically, therefore, one cannot conclude with certainty that the degrees of blanching induced by the two formulations are sufficiently different over the peak observation times to ascribe inequivalence to their drug delivery patterns.

The quantitative data of the in vitro assessment are amenable to normal statistical treatment and may be compared by simple Student's t-distribution test methodology. As expected, there were no significant differences found, at the 95% level of significance, in the drug permeation from the two formulations at any sampling time. Statistically, therefore, the drug delivery from the two products is equivalent when using this in vitro methodology.

IV. CONCLUSIONS

Good correlation of the in vitro permeation data with the in vivo blanching results is, thus, demonstrated in this investigation. The diffusion cell permeation data monitored over 72 h indicates no significant differences in the drug release rates of the two hydrocortisone formulations, and, in congruity, the in vivo blanching data monitored over 28 h indicate that equivalent degrees of blanching are elicited by the two products. These methodologies, therefore, are complimentary to one another and may be used in combination for topical equivalence testing or quality control procedures. The availability of correlating systems such as this is becoming more important in topical drug absorption research, especially in regulatory affairs, for the registration of new products. It must be stressed, however, that the correlation observed here has been validated only for the two products compared in this study; there is no implication that this correlation will extend to the entire family of hydrocortisone products. With the diversity of topical delivery vehicles possible with technology available today, each formulation to be tested in this manner would require a fully validated in vitro protocol.16

References

4. Wend, H. and Frosch, P.J., Clinico-pharmacological Models for the Assay of Topical Corticosteroids, Karger, Basel, 1982.