Strategies for the Bioequivalence Assessment of Topical Dosage Forms

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BIOAVAILABILITY

- Systemically absorbed products
- Products not intended to be absorbed

...... the rate and extent to which the active ingredient or moiety is absorbed from the drug product and becomes available at the site of action.

21 CFR 320.1(a)

...... may be assessed by (surrogate) measurements intended to reflect the rate and extent to which the active ingredient or moiety becomes available at the site of action.
Products Intended to be Absorbed into Systemic Circulation

- Surrogate measures justified by the presumption that concentration of drug in blood stream is in equilibrium and reflects the concentration at site of action.
- Relationship between effectiveness and systemic blood concentrations of drug implied.

Topical Products Not Intended to be Absorbed

- Surrogate measures cannot be justified on same basis as for drugs intended to be absorbed.
- No such relationship expected.
Products Intended to be Absorbed into Systemic Circulation

- Methodology well established
- Statistical assessment of data well established
- Regulatory requirements based up $C_{max}$ and AUC falling within prescribed limits of CI of 90% and relative means of test to reference being within 80-125%

Topical Products Not Intended to be Absorbed

- Methodology under development
- Statistical assessment yet to be defined
- Regulatory requirements?

*except for topical dermatologic corticosteroids where the FDA Guidance requires that Locke’s method, which provides an exact confidence interval from untransformed data, be used.*
Bioequivalence Assessment (Safety and Efficacy)

- Clinical Measures
  Patient Studies

- Surrogate Measures
  Patients or Healthy Human Subjects
• **TOPICAL**
  
  “Belonging to a Place or Spot”
  Topical = Local

  **TRANSDERMAL** products for treatment of systemic diseases

  Aimed at achieving systemically active drug concentrations

  Percutaneous absorption is a prerequisite for activity

  Ideally, no local drug accumulation
G. L. Flynn and N. D. Weiner in Dermal and Transdermal Drug Delivery, p. 37, 1989
TRANSDERMAL DOSAGE FORMS

• Dosage route capable of avoiding presystemic or first-pass metabolism
• Convenient to use with improved patient compliance
• Provides systemic blood levels that are therapeutically efficacious

NB: Must be NON-IRRITATING and NON-SENSITIZING and must adhere to the site of application for the entire delivery time

The drug delivery must overcome a series of barriers within the skin to reach the systemic circulation
FIGURE 1  Schematic of drug permeation through the main skin barrier, the Stratum Corneum, to the vascularized viable epidermis and dermis. The disposition of the drug after absorption from the transdermal system is also illustrated.
In Vivo Requirements i.e. Bioequivalence Assessment

- Conduct single dose study in healthy human volunteers
- Compare the generic product (Test) with the RLD
- Use a randomized, 2 period, 2 treatment cross-over study
- Statistical analysis based on average bioequivalence

Acceptance Criteria:
$C_{\text{max}}, \ AUC_{0-t}$ where $t=$ last sampling time, $AUC_{0-t + 1 \text{ hr}}$
CI for ln-transformed $C_{\text{max}}$ and AUCs must fall within 80% -125%
WEAR, SKIN IRRITATION AND SENSITIZATION STUDIES

FDA Guidance . Skin Irritation and Sensitization Testing of Generic Transdermal Drug Products, December 1999
RECALLED

Wear and Apparent Dose:
i.e. define the wear and adhesion properties of the test product

• Compare T & R in a single dose, randomized, 2 way cross-over study
• N = 50 and a 1 week washout between treatments
• Check for adherence after application at various time intervals
# ADHESION ASSESSMENT

<table>
<thead>
<tr>
<th>Adhesion Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>TDS adhered &gt; 90% (Essentially no lift off the skin)</td>
</tr>
<tr>
<td>1</td>
<td>TDS adhered &gt; 75% to &lt; 90% (only some of the edges lifting off the skin)</td>
</tr>
<tr>
<td>2</td>
<td>TDS adhered &gt; 50% to &lt; 75% (&lt;50% lifting off the skin)</td>
</tr>
<tr>
<td>3</td>
<td>TDS adhered &lt; 50% but not detached (&gt;50% lifting off the skin without falling off)</td>
</tr>
<tr>
<td>4</td>
<td>TDS detached (Completely off the skin)</td>
</tr>
</tbody>
</table>

After removal of each TDS, application site examined for evidence of local skin irritation
<table>
<thead>
<tr>
<th>Score</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No evidence of irritation</td>
</tr>
<tr>
<td>1</td>
<td>Minimal erythema (barely perceptible)</td>
</tr>
<tr>
<td>2</td>
<td>Definite erythema (readily visible) with minimal edema or papular response</td>
</tr>
<tr>
<td>3</td>
<td>Erythema with papules</td>
</tr>
<tr>
<td>4</td>
<td>Definite edema</td>
</tr>
<tr>
<td>5</td>
<td>Erythema, edema and papules</td>
</tr>
<tr>
<td>6</td>
<td>Vesicular eruption</td>
</tr>
<tr>
<td>7</td>
<td>Strong reaction spreading beyond test site</td>
</tr>
</tbody>
</table>

After removal of the TDS, the amount of adhesive remaining at the application site is examined and graded: 0 (none), 1 (light), 2 (medium) and 3 (heavy)
LOSS OF DRUG FROM TDS

- Test each TDS unit removed for drug content
- Loss of drug = apparent dose i.e.
  Apparent Dose = Initial Potency – Residual Potency

The above useful to define dose delivered by the TDS for labelling purposes.
TOPICAL products for cutaneous (dermatologic) use

Pharmacologic or other effect confined to surface of skin or within the skin

May or may not require percutaneous penetration and deposition
REGIONAL products for treatment of disease or symptoms in deeper tissue, e.g. topical anti-inflammatory products

Aimed at achieving systemically active drug concentrations

Percutaneous absorption is a prerequisite for activity

Ideally, no local drug accumulation
G. L. Flynn and N. D. Weiner in Dermal and Transdermal Drug Delivery, p. 37, 1989
The **Human Skin Blanching Assay** (HBSA) *aka* the Vasoconstrictor Assay (VCA) for assessing topical corticosteroid products

1st observed in 1950 *(Hollander et al)*

McKenzie & Stoughton – 1962

Indirect measure using a supposed vasoconstriction response following application of topical corticosteroid to skin
A typical blanching response after the application of some topical corticosteroid formulations.
METHODOLOGY

instrumental (chromameter or visual (human eye) ?

• Application to a number of skin sites
• Fixed contact time (dose duration)
• Excess product removed by gentle washing
“In an era with increasingly sophisticated methods to detect changes in light, temperature, pressure and other physical and chemical changes, the use of a human observer to assess the magnitude of a pharmacodynamic effect becomes increasingly inadequate. Application of a commercially available chromameter (or colorimeter; e.g. Chroma Meter 200 or 300 model series, Minolta) to detect erythema offers the possibility of replacing subjective visual scoring in the vasoconstrictor assay with objective, quantifiable measurements. The Division of Bioequivalence currently considers the use of a chromameter to be applicable to bioequivalence studies based on the vasoconstrictor assay, and therefore recommends that pharmaceutical sponsors incorporate the use of a chromameter into their study designs.”
FDA Guidance Suggests:

• Conducting two *in vivo* studies - a pilot dose duration - response study and a pivotal bioequivalence study comparing test and reference products.

• The $ED_{50}$ is determined in the pilot study and the comparison of test and reference products in the pivotal study is conducted as a dose duration approximately equal to the population $ED_{50}$. 
Determination of Dose Duration

- Pilot study required to determine dose duration.
  - $E_{\text{max}}$ model - most discriminatory dose duration

$$E = E_0 + \frac{E_{\text{max}} \times D}{ED_{50} + D}$$

where $E$ = effect elicited
$E_0$ = baseline effect in the absence of ligand
$E_{\text{max}}$ = maximum effect elicited
$ED_{50}$ = dose duration (D) at which effect is half-maximal
The FDA Guidance recommends that a subject must be a ‘detector’ in order for inclusion of their data for statistical analyses in the bioequivalence assessment.

Hence, subjects’ responses are expected to meet the specified minimum $D_2/D_1$ ratio of AUEC values in the pivotal study as shown in the equation below.

\[
\frac{\text{AUEC at } D_2}{\text{AUEC at } D_1} \geq 1.25
\]

where $D_1 = \frac{1}{2}ED_{50}$ and $D_2 = 2ED_{50}$
Tape Stripping – a dermatopharmacokinetic approach


- Initial TS methodology outlining the bioavailability/bioequivalence protocol for topical formulations intended for local and/or regional activity, published in a draft guideline

- subject to criticism which resulted in its withdrawal, mainly due to a number of limitations, in particular the sources of variability and control
• Dermatopharmacokinetic approach
• Determines the amount of drug permeated into the *stratum corneum*
• Utilizes adhesive tape strips Transpore, Micropore, Scotch, D-Squame tapes
• Relatively non-invasive
• Removes layers of *stratum corneum*
Tape stripping process
• TS is relatively non-invasive
• However, *stratum corneum* thickness differs between each individual – hence, normalization necessary - measure transepidermal water loss (TEWL):

\[
1/J = 1/\text{TEWL}_x = H-x/K.D.\Delta C
\]

- \( J \) = flux \( \text{g/m}^2\text{h} \)
- \( H \) = total SC thickness
- \( x \) = partial SC thickness
- \( K \) = partition coefficient of water in tissue
- \( D \) = water diffusivity
- \( \Delta C \) = difference in water concentration across the membrane

• To determine bioequivalence of Dermovate creams using HSBA and also tape stripping

• Investigate whether tape stripping can show differences in bioavailability between the same and different topical products, i.e. the capability to measure bioequivalence or bio-inequivalence
• Pivotal TS study - 30 subjects

• Same Dermovate® cream as the test and reference product in the pivotal HSBA study

• Dovate® cream vs Dermovate® cream

• Dermovate® ointment vs Dermovate® cream
Bioequivalence assessment of identical products (test – Dermovate® cream, reference – Dermovate® cream)

<table>
<thead>
<tr>
<th></th>
<th>Mean T/R ratio (%)</th>
<th>90% CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Locke’s</td>
<td>Log-transformed</td>
</tr>
<tr>
<td>HSBA (n=34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromameter</td>
<td>104.3</td>
<td>90.2-120.7</td>
</tr>
<tr>
<td>Tape stripping</td>
<td>101.8</td>
<td>87.4-117.7</td>
</tr>
<tr>
<td>Study Description</td>
<td>T/R ratio (%)</td>
<td>Confidence interval (%)</td>
</tr>
<tr>
<td>-------------------------------------------------------</td>
<td>---------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td><strong>Pivotal TS Study (n=30)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dovate® cream vs Dermovate® cream</td>
<td>92.4</td>
<td>80.3 – 106.0</td>
</tr>
<tr>
<td>Dermovate® ointment vs Dermovate® cream</td>
<td>59.1</td>
<td>49.3 – 70.2</td>
</tr>
</tbody>
</table>

- Reference product: Dermovate® cream
- Test products: Dovate® cream and Dermovate® ointment

Comparison of Tape Stripping with the Human Skin Blanching Assay for the Bioequivalence Assessment of Topical Clobetasol Propionate Formulations
Tape stripping for the BE of clotrimazole

Ref: Bioequivalence of Topical Clotrimazole Formulations: An Improved Tape Stripping Method
Natalie Rae Parfitt, Michael Skinner, Charles Bon, Isadore Kanfer

J Pharm Pharmaceut Sci (www.cspscanada.org) 14(3) 347 - 357, 2011
<table>
<thead>
<tr>
<th></th>
<th>Untransformed Data</th>
<th>Transformed Data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td><strong>$\frac{AUC_{\text{test}}}{AUC_{\text{reference}}}$</strong></td>
<td>0.94</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>CI 90%</strong></td>
<td><strong>0.82 - 1.08</strong></td>
<td><strong>0.82 - 1.13</strong></td>
</tr>
<tr>
<td>Bioequivalence (0.8-1.25)?</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>CV%</strong></td>
<td>23.62%</td>
<td>23.40%</td>
</tr>
<tr>
<td><strong>Power</strong></td>
<td>n/d</td>
<td>47.24%</td>
</tr>
<tr>
<td><strong>n required for 80% power</strong></td>
<td>19</td>
<td>21</td>
</tr>
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## Effect Of Widening The Bioequivalence Limits

<table>
<thead>
<tr>
<th>Bioequivalence limits</th>
<th>Sample size required for 80 % power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untransformed data</td>
</tr>
<tr>
<td>0.8 – 1.25</td>
<td>19</td>
</tr>
<tr>
<td>0.75 – 1.33</td>
<td>14</td>
</tr>
<tr>
<td>0.7 – 1.43</td>
<td>&lt;13</td>
</tr>
</tbody>
</table>
### Bioequivalence of clotrimazole cream vs clotrimazole gel

<table>
<thead>
<tr>
<th></th>
<th>Transformed</th>
<th>Untransformed</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>$\frac{AUC_{\text{test}}}{AUC_{\text{reference}}}$</td>
<td>1.67</td>
<td>2.06</td>
</tr>
<tr>
<td>CI 90%</td>
<td><strong>0.91 - 3.23</strong></td>
<td><strong>1.06 - 3.99</strong></td>
</tr>
<tr>
<td>Bioequivalence? (0.8 – 1.25)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CV%</td>
<td>27.74 %</td>
<td>24.61 %</td>
</tr>
</tbody>
</table>
Bioequivalence of Topical Clotrimazole Formulations: An Improved Tape Stripping Method
Natalie Rae Parfitt, Michael Skinner, Charles Bon, Isadore Kanfer
J Pharm Pharmaceut Sci (www.cspscCanada.org) 14(3) 347 - 357, 2011
• Microdialysis (MD) - *in vivo* sampling technique to measure endogenous and/or exogenous compounds in extracellular spaces

• Dermal Microdialysis (DMD) is a relatively new application of MD which allows continuous monitoring of endogenous and/or exogenous solutes in the interstitial fluid (ISF) of dermal tissue with minimal tissue trauma

• The technique involves the implantation of a semi-permeable membrane into a specific region of a tissue or fluid-filled space
Anatomy and physiology of the skin with the potential target or sites of action of selected analgesics

Implantation of linear DMD probes in the skin.

A: Guide cannula insertion at the entry point marked on the skin.

B: Guide cannula pierced through the exit point and MD probes inserted into the guide cannula.

C: Guide cannula withdrawal leaving the MD probe within the dermis.
## Bioequivalence of a 2.5 % m/m ketoprofen gel

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Arithmetic means (mean±S.D.)</th>
<th>%Ratio (S1/S2)</th>
<th>90%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A) AUC(_{0-5})</td>
<td>155.51</td>
<td>149.98</td>
<td>106.16</td>
</tr>
<tr>
<td>(B) AUC(_{0-5})</td>
<td>152.04</td>
<td>153.45</td>
<td>99.01</td>
</tr>
<tr>
<td>(C) AUC(_{0-5})</td>
<td>139.89</td>
<td>165.6</td>
<td>86.69</td>
</tr>
</tbody>
</table>

**SEQUENCES**

A (TTRR/RRTT), B (TRTR/RTRT) and C (TRRT/RTTR).
Application of dermal microdialysis for the evaluation of bioequivalence of a ketoprofen topical gel
Ralph Nii Okai Tettey-Amlalo, Isadore Kanfer, Michael F. Skinner, Eva Benfeldt, Roger K. Verbeeck
Currently only “surrogate” measure for the assessment of bioequivalence of topical products “officially” recognised – HSBA for topical corticosteroids

Tape stripping data provided the same results as that of the HSBA i.e. correlation between the two methods

Tape stripping shown to be a viable alternative BE method for the assessment of topical preparations, e.g., clotrimazole and other topical antifungals and in general?

Dermal microdialysis – potential for use for BE assessment of some topical products, e.g., ketoprofen and other topical NSAIDS
Acknowledgements

• Rhodes University – Labs & Facilities
• Funding from the National Research Foundation
• Graduate students: (Topical Medicines Research Program)
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  N. Mandimika-(TS & DMD)
  R. Tettey-Amlalo-(TS & DMD)
  W-L Au-(HSBA, TS & DMD)
  N. Parfitt-(TS)
• Post-Doctoral Fellow. Dr. S. S. R. Patnala (Pharm.Anal.)
• Research Collaborators:
  Dr. M. Skinner – Biopharmaceutics Research Institute, Rhodes University, South Africa - (Topical Medicines Research Program)
• Dr. Eva Benfeldt, University of Copenhagen (DMD)
• Prof. Roger Verbeeck, Catholic University of Louvain in Belgium (DMD)
• Chuck Bon, Biostudy Solutions, USA (TS)
• THANK YOU for your attention.