Characterizing In Vitro Bioavailability of Acyclovir and Metronidazole Topical Products, and In Vitro – In Vivo Correlation Results with Transdermal Systems

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The views expressed in this presentation do not reflect the official policies of the U.S. Food and Drug Administration or the U.S. Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.
Topical Dose Administration Techniques

• Highly variable among labs, researchers, and patients
  • Methods of dispensing formulation
  • Duration of rubbing
  • Force used for rubbing
  • Loss of formulation during rubbing

• Need a reproducible, clinically-relevant, and practical technique for IVPT

Image from http://www.telegraph.co.uk/expat/expatlife/10441983/Pale-and-interesting.html
IVPT Results Variability
Importance of Dose Application – Voltaren® gel example
Dose Test and Reference Products the Same

<table>
<thead>
<tr>
<th>Dose (mg/cm²)</th>
<th>J_{\text{max}} \pm \text{SD (µg/cm²/h)}</th>
<th>T_{\text{max}} (h)</th>
<th>Cumulative Amount \pm \text{SD (µg/cm²)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>2.29 ± 0.57</td>
<td>8</td>
<td>24.91 ± 3.38</td>
</tr>
<tr>
<td>10</td>
<td>0.48 ± 0.19</td>
<td>4</td>
<td>6.10 ± 0.61</td>
</tr>
</tbody>
</table>

HPLC vial rubbing application technique
**IVPT Results Variability**

**Importance of Dose Application – Pennsaid® 2%**

**Dose Test and Reference Products the Same**

<table>
<thead>
<tr>
<th>Dose (mg/cm²)</th>
<th>J&lt;sub&gt;max&lt;/sub&gt; ± SD (µg/cm²/h)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>Cumulative Amount ± SD (µg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/cm²</td>
<td>4.05 ± 1.06</td>
<td>24</td>
<td>45.79 ± 3.00</td>
</tr>
<tr>
<td>5 mg/cm²</td>
<td>4.59 ± 1.09</td>
<td>6</td>
<td>39.43 ± 3.90</td>
</tr>
</tbody>
</table>

HPLC vial rubbing application technique
Dose Administration Techniques

Positive Displacement Pipette

- Quick, convenient, low variability
- Minimal formulation loss
- Lack of rubbing effect

Inverted HPLC Vial

- Time-consuming, more variability
- Some formulation loss
- Simulates clinically-relevant rubbing effect
**Dose Administration Techniques**

**U.S. Zovirax®**

- Positive Displacement Pipette
- Inverted HPLC Vial

**U.K. Zovirax®**

- Ex vivo human skin
- Mean ± SD (n=3-4 for each technique)
Preliminary: Dose Administration Techniques

Pennsaid® 2% (more viscous)

Orange Arrow: dosing (~5 mg/cm² of formulation)

Mean ± SD (n=3-4)
Yucatan Miniature Pig Skin
Four Acyclovir Cream Products

(Mean ± SEM, n= 6 donors with 4-7 replicates per donor for Zovirax® creams and n = 2 donors with 3-4 replicates per donor for non-Zovirax® creams)

**The IVPT method was able to discriminate the Reference and Test acyclovir products, based on Jmax and the total amount of acyclovir permeated over 48 h**

Positive displacement pipette application
**J**\textsubscript{max} and the total amount of acyclovir permeated over 48h between Reference and Test

<table>
<thead>
<tr>
<th>U.S. Zovirax®</th>
<th>U.K. Zovirax®</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>J</strong>\textsubscript{max} (µg/cm²h)</td>
<td>**Total Permeation (µg/cm²)</td>
</tr>
<tr>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.05</td>
<td>1.0</td>
</tr>
<tr>
<td>0.10</td>
<td>2.0</td>
</tr>
<tr>
<td>0.15</td>
<td>3.0</td>
</tr>
<tr>
<td>0.20</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* \((p=0.0384)\)

** \((p=0.0083)\)

Positive displacement pipette application

Comparisons of products (Mean ± SEM, n= 6 donors with 4-7 replicates per per donor)
U.S. vs. U.K. Zovirax® creams per donor

Positive displacement pipette application
Metronidazole RLD Gel & Generic vs. Generic Cream

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Cumulative Cutaneous Absorption (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLD Gel (n=3)</td>
<td>8.93 ± 2.33</td>
</tr>
<tr>
<td>Generic Gel (n=3)</td>
<td>9.70 ± 2.42</td>
</tr>
<tr>
<td>Generic Cream (n=3)</td>
<td>21.0 ± 10.32</td>
</tr>
</tbody>
</table>

Dosing Technique: Inverted HPLC vial
Target dose: 10 mg/cm²
Flow rate: 1.0 mL/h
Skin surface temperature: 32 ± 2°C (circulating water bath)
Receiver solution: Isotonic phosphate buffer (pH 7.4 ± 0.1)
Skin: human abdominal skin from three donors with four replicate skin sections per donor per product

Cumulative absorption from RLD gel, generic metronidazole gel and generic metronidazole cream over 24-h study duration.
## Metronidazole RLD Gel & Generic vs. Generic Cream

<table>
<thead>
<tr>
<th>Product</th>
<th>Maximum Flux (μg/cm²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLD Gel (n=3)</td>
<td>0.93 ± 0.63</td>
</tr>
<tr>
<td>Generic Gel (n=3)</td>
<td>1.22 ± 0.69</td>
</tr>
<tr>
<td>Generic Cream (n=3)</td>
<td>Observed at ≥ 12 h</td>
</tr>
</tbody>
</table>

**Flux vs Time**

Graph showing flux over time with RLD gel, generic metronidazole gel, and generic metronidazole cream.

Flux profile from RLD gel, generic metronidazole gel and generic metronidazole cream over 24-h study duration.
Conclusion: Metronidazole IVPT results

• IVPT studies may have utility to help support an evaluation of bioequivalence for topical drug products
  – RLD and generic gels
    • Positive controls for bioequivalence relative to each other
    • Had a similar rate and extent of metronidazole delivery
    • Discriminated the cutaneous bioavailability from the cream as being different from that for both gels
  – Generic cream
    • Negative control for bioequivalence relative to the reference gel
    • Distinct rate and extent of metronidazole delivery with respect to both gels

• Consistent with the expectation that differences in physical and structural critical quality attributes between topical semisolid drug products (e.g., between a gel and a cream) can alter the bioavailability of metronidazole

Qingzhao Zhang PhD Candidate, AAPS Poster 2017, Human PK Study Pending
Can the in vitro permeation test (IVPT) predict the performance of TDS (patch) and heat effects on drug delivery and absorption in vivo?

Model Drugs: **Nicotine** & Fentanyl

I. Evaluation of the influence of transient heat (1 h) on the release and permeation of drug from TDS using the *in vitro* permeation test (IVPT)

II. Evaluation of the influence of transient heat (1 h) on the TDS pharmacokinetics *in vivo* by conducting PK studies in human subjects

III. Evaluation of preliminary *in vitro* and *in vivo* correlations (IVIVC) of TDS

*This TDS project is informative for topical drug product evaluation since many provide quantifiable blood levels of drug
Temperature Monitoring & Heat Application *In Vitro*

Infrared Thermometer

Temperature Monitoring & Heat Application *In Vivo*

- Kevlar sleeve with an opening to expose TDS, while protecting skin outside the dosing area
- Thermometer probe adjacent to TDS
- Pre-heated heating pad
- ACE™ Bandage to ensure good contact between TDS and heating pad

Thermometer image from http://static.coleparmer.com/large_images/91427_10_5.jpg
Temperature: *In Vitro* & *In Vivo*

- Early Heat - *In Vitro*
- Late Heat - *In Vitro*
- Early Heat - *In Vivo*
- Late Heat - *In Vivo*

(42 ± 2°C)
IVPT Results

Human Skin Data

Mean ± SEM from 4 donors for Early Heat and Late Heat, 2 donors for Baseline with n=4 per donor

Flux+ = Flux value multiplied by TDS size to account for the whole TDS

Two-way ANOVA followed by Bonferroni’s post-hoc multiple comparisons
In Vivo Results

**NicoDerm CQ®**
- Early Heat
- Late Heat

**Aveva**

Mean ± SD from 10 human subjects

Smokers
Patch off 9h

Two-way ANOVA followed by Bonferroni’s post-hoc multiple comparisons
Conclusions – Nicotine

• Early vs. Late Heat effect comparable both *in vitro* and *in vivo*

• Heat effect on two differently formulated TDS comparable both *in vitro* and *in vivo*

• *In vitro* and *in vivo* heat effect ratios were comparable

• Strong preliminary IVIVCs (IVIVRs) between IVPT and clinical human PK studies under the matched study designs
IVIVC

• Definition by the U.S. FDA
  “a predictive mathematical model describing the relationship between an in-vitro property of a dosage form and an in-vivo response”

➤ **Level A**: a point-to-point correlation between in vitro and in vivo profiles

➤ **Level B**: comparison between in vitro dissolution time and in vivo residence time

➤ **Level C**: a single point correlation between in vitro and in vivo parameters (e.g. $J_{\text{max}}$ vs. $C_{\text{max}}$)
Approach I

Level A

Eq. 1 prediction while TDS was worn:

\[ C_s = \frac{F \times R_{in} \times H_i}{CL_{IV}} \times (1 - e^{-k_1 t}) \]

Eq. 2 prediction after TDS removal:

\[ C_s = C_0 \times e^{-k_2 t} \]

Or may need 2 or 3 compartment model

Depending on drug and available data

\[ C_s: \] Predicted in vivo serum concentration

\[ F: \] Absolute bioavailability for TDS

\[ R_{in}: \] Rate of input (mean flux during steady-state in IVPT experiments)

\[ H_i: \] In vitro heat effect coefficient (composite heat effect during and after heat exposure); ratio of flux values with heat and without heat

\[ CL: \] Total body clearance obtained from literature/product package information

\[ k: \] Elimination rate constant obtained from literature/product package information

\[ k_1: \] after IV dose; \( k_2: \) after TDS dose

\[ (k_1: \] a derived PK parameter from the two fundamental PK parameters (Cl and V). \( k_1 = \frac{Cl}{V}. \) \( k_1 \) is a re-parameterization of Cl and V

\[ F \times R_{in} \] is used to mimic an IV dose and as a result Cliv is used. Therefore Kiv (Cliv/V)

\[ t: \] Time after administration of TDS for Eq.1 and time after removal of TDS for Eq. 2

\[ C_0: \] Initial concentration after TDS removal
**Approach II and III**

1. Reconstruct baseline (without heat) profile by combining non-heat portion from two study designs

2. Deconvolute in vivo baseline conc. vs time profile using Phoenix®

3. Construct IVIVC model by plotting fraction permeated in vitro vs. fraction absorbed in vivo

4. Predict in vivo fraction absorbed using the IVIVC model and IVPT data

5. Convolute the predicted in vivo fraction absorbed data using Phoenix® to obtain conc. vs. time profile

6. Apply **in vitro** heat effect coefficient, \( H_i \) (Approach II) or **in vivo** heat effect coefficient, \( H_{ii} \) (Approach III) to the predicted in vivo profile
Approach I

**NicoDerm CQ® - Early Heat**

- Predicted
- Observed Mean±SD (n=10)

**Aveva - Early Heat**

- Predicted
- Observed Mean±SD (n=10)

**NicoDerm CQ® - Late Heat**

- Predicted
- Observed Mean±SD (n=10)

**Aveva - Late Heat**

- Predicted
- Observed Mean±SD (n=10)
Approach II

in vitro heat effect coefficient, Hi
Approach III

in vivo heat effect coefficient, $H_{ii}$
## % Prediction Error

<table>
<thead>
<tr>
<th>Nicotine TDS</th>
<th>NicoDerm CQ®</th>
<th>Aveva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early Heat</td>
<td>Late Heat</td>
</tr>
<tr>
<td><strong>Approach I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AUC</td>
<td>20.3</td>
<td>12.9</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>14.4</td>
<td>16.6</td>
</tr>
<tr>
<td><strong>Approach II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AUC</td>
<td>10.3</td>
<td>5.0</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>23.3</td>
<td>30.2</td>
</tr>
<tr>
<td><strong>Approach III</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AUC</td>
<td>5.1</td>
<td>1.2</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>15.0</td>
<td>5.8</td>
</tr>
</tbody>
</table>
Fentanyl IVPT Results

Mean ± SEM from 4 donors with n=4 per each donor

**Duragesic®**

**Apotex**

**Mylan**

Flux+ = Flux value multiplied by TDS size to account for the whole TDS

Two-way ANOVA followed by Bonferroni’s post-hoc multiple comparisons
Fentanyl Results

In Vitro
Mean ± SEM from 4 donors with n=4 per donor (Human Skin)

Duragesic®

Flux+ (μg/hr)

Time (h)

In Vivo
Mean ± SD from 10 Healthy Adults

Mylan

Flux+ = Flux value multiplied by TDS size to account for the whole TDS

Apotex

Flux+ (μg/hr)

Time (h)

Flux+ (μg/hr)

Time (h)
## Clearance Value of Fentanyl

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subject #</th>
<th>Condition</th>
<th>$\text{CL}_{IV}$ (L/h)</th>
<th># of comp for PK Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ariano et al. J Clin Pharmacol 2001</td>
<td>18</td>
<td>Healthy</td>
<td>128</td>
<td>1</td>
</tr>
<tr>
<td>Bower et al. Br J Anaesth 1982</td>
<td>7</td>
<td>Healthy</td>
<td>92</td>
<td>2</td>
</tr>
<tr>
<td>Bentley et al. Anesth Analg 1982</td>
<td>5</td>
<td>Surgical</td>
<td>59</td>
<td>3</td>
</tr>
<tr>
<td>Varvel et al. Anesthesiology 1989(^1)</td>
<td>8</td>
<td>Surgical</td>
<td>46</td>
<td>3</td>
</tr>
<tr>
<td>Shibutani et al. Anesthesiology 2004</td>
<td>16</td>
<td>Surgical</td>
<td>43</td>
<td>3</td>
</tr>
<tr>
<td>Haberer et al. Br J Anaesth 1982</td>
<td>13</td>
<td>Surgical</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>Scott et al. J Pharmaol Exp Ther 1986</td>
<td>15</td>
<td>Healthy</td>
<td>34</td>
<td>2</td>
</tr>
<tr>
<td>Univ. of Maryland, Baltimore (ongoing)</td>
<td>14</td>
<td>Healthy</td>
<td>11</td>
<td>2</td>
</tr>
</tbody>
</table>

Weighted Mean $\text{CL}_{IV}$ from Healthy subjects with PK value obtained from 2 or 3 compartmental analysis = 33.6 L/h

\(^1\) Source of IV PK parameters reported in Duragesic® Package Insert
Approach I

Grey shade represents prediction range when inter-subject variability of CL = 50%
Approach I

Grey shade represents prediction range when inter-subject variability of CL = 50%
Subject TDF 024: Predicted using the subject’s own F, CL_{IV} and k values

Approach I
Approach II

Duragesic® - Early Heat
- Predicted (Approach II)
- Observed In Vivo (n=10)

Duragesic® - Late Heat

Apotex - Early Heat

Apotex - Late Heat

Mylan - Early Heat

Mylan - Late Heat
## % Prediction Error

<table>
<thead>
<tr>
<th>Fentanyl TDS</th>
<th>Duragesic®</th>
<th>Apotex</th>
<th>Mylan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early Heat</td>
<td>Late Heat</td>
<td>Early Heat</td>
</tr>
<tr>
<td><strong>Approach I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AUC</td>
<td>31.7</td>
<td>17.5</td>
<td>4.0</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>37.7</td>
<td>36.8</td>
<td>29.8</td>
</tr>
<tr>
<td><strong>Approach II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AUC</td>
<td>3.3</td>
<td>13.1</td>
<td>10.2</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>23.4</td>
<td>23.6</td>
<td>39.6</td>
</tr>
<tr>
<td><strong>Approach III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AUC</td>
<td>15.2</td>
<td>10.1</td>
<td>11.9</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>0.5</td>
<td>2.3</td>
<td>4.4</td>
</tr>
</tbody>
</table>
Conclusions – Fentanyl

• Early vs. Late Heat effect comparable both *in vitro* and *in vivo*

• Heat effect on three differently formulated TDS comparable both *in vitro* and *in vivo*

• However, *in vivo* heat effect seemed to be higher compared to the *in vitro* heat effect

• Preliminary IVIVCs between IVPT and clinical human PK studies under the matched study designs
  ⇒ Not as predictive compared to nicotine...

😊 Why??
1. Lipophilicity of Fentanyl

Nicotine TDs

- NicoDerm CQ®
- Aveva

$t_{1/2} \sim 2-3 \text{ h}$

After TDS Removal

Fentanyl TDS

- Duragesic®
- Apotex
- Mylan

$t_{1/2} \sim 20-27 \text{ h}$

After TDS Removal
2. High Inter-subject Variability of Fentanyl

Heat Effect Ratio was determined by the ratio of the $C_{\text{max}}$ during the 3h window and the concentration immediately before heat application.
Conclusions - IVIVC

• Three approaches were evaluated to demonstrate a preliminary Level A IVIVC (IVIVR) for TDS

• Good preliminary IVIVC demonstrated for nicotine TDS, including heat effect

• Weaker preliminary IVIVC found for fentanyl TDS
  • Limitation of mimicking drug reservoir in skin layers, microcirculation and subcutaneous tissue in vitro
  • High inter-subject variability for fentanyl (+ Lack of reliable PK parameters)
Take Home Messages

• An *in vitro* heat effect study may be able to predict the *in vivo* heat effect for some drugs, following an IVIVC validation

• For certain drugs, an *in vivo* heat factor may need to be determined

• Heat effects are drug molecule and formulation excipient dependent---Diclofenac formulation data not shown

• Patches are not the only topical products affected by heat
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- Dani Fox (Clinical Coordinator)
- Sagar Shukla (Lidocaine)
- Paige Zambrana (Sunscreens & glucose monitoring)
- Qingzhao Zhang (Metronidazole & rivastigmine)
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