Drug Permeability: Best Practices for BCS-based Biowaivers

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M-CERSI workshop
December 6, 2021
Agenda

- Establishment of in-house validated *in vitro* models for permeability
  - Caco-2
  - Intestinal animal tissue
- Case studies
# Biowaiver guideline changes

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2017</th>
<th>2021</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCS</strong></td>
<td>1 &amp; 3</td>
<td></td>
<td>Different salts for BCS1 allowed</td>
</tr>
<tr>
<td><strong>Dosage form</strong></td>
<td>IR solid oral Not for narrow therapeutic window Not for products absorbed in oral cavity</td>
<td></td>
<td>Suspending Only when mode of administration includes water</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Highest strength Soluble in 250 mL or less @ pH 1 - 6.8 @ 37° ± 1°C; 3 replicates or more pH check (start/rend) Shake-flask, acid-base titrations Degradation of drug should be reported</td>
<td>Additional information needed when the highest single does is not the same as the highest strength – linearity of PK</td>
<td>Highest single therapeutic dose Solubility maintained over relevant timeframes to accommodate expected duration of absorption Smaller volumes allowed NMT 10% degraded</td>
</tr>
<tr>
<td><strong>Papp</strong></td>
<td>$F_{app}$ in humans Rate&amp;mass transfer through human intestinal membrane Perfusion studies on humans Alternative models for passive permeation: in situ/in vivo animal, in vitro epithelial cell cultures Model suitability demonstration More than one method may be used when conflicting evidence $F_{app}$ ≥ 85 % or ≥ 85 % unchanged drug recovered in urine/recovered in urine as drug + metabolites</td>
<td>Mass recovery for in vitro models &gt; 80 %</td>
<td>$F_{app}$ ≥ 85 % ≥ 85 % unchanged drug recovered in urine/recovered in urine as drug + Phase I, phase II metabolites For mass balance: metabolites from feces, if they were formed after absorption; unchanged drug secreted by biliary route, intestinal secretion or from unstable metabolite converted back to drug by microbiota. Only Caco-2 in vitro model allowed.</td>
</tr>
<tr>
<td><strong>Stability</strong></td>
<td>Stability in the GI tract unless ≥ 85 % uncaged drug recovered in urine In vivo/in situ perfusion methods that are based on measuring the loss of drug from application site Aspirate HGF/HIF or SGF/SIF, incubate @ 37°C for 1h/3h; NMT 5% degradation</td>
<td>Required for mass balance studies and for Caco-2 studies Degradation NMT 10%</td>
<td></td>
</tr>
<tr>
<td><strong>Disso</strong></td>
<td>AP1, 100 rpm or AP2, 50 rpm (75 rpm when justified, coning) 500 mL or less (0.1N HCl or SGF, pH 4.5, pH 6.8 or SIF) Rapidly dissolving: NLT 85% @ 30 min Very rapidly dissolving: NLT 85% @ 15 min</td>
<td>900 mL or less; AP1, 100 rpm or AP2, 50 rpm, Coning BCS 1: test and reference both either ≥ 85% @ 15 min or @ 30 min, or use $f_2$</td>
<td></td>
</tr>
<tr>
<td><strong>Excipients</strong></td>
<td>Do not affect rate or extent of absorption for BCS 1, new/atypical excipients, high quantities, surfactants, sweeteners BCS 3: qualitatively the same, quantitatively very similar</td>
<td>Criteria of BCS 3 quantitative changes in excipients listed based on excipient role</td>
<td>Amount of excipient, the mechanism by which it could affect absorption and drug absorption properties Slowly absorbed BCS 1 Qualitative and quantitative differences allowed (± 10 %)</td>
</tr>
<tr>
<td><strong>Prodrugs</strong></td>
<td>$P_{app}$ determined for species that is absorbed Solubility for both important</td>
<td></td>
<td>Only when absorbed as prodrugs</td>
</tr>
</tbody>
</table>
Validation of in-house Caco-2 (and rat intestine model)

Figure 5. Correlation of human fraction absorbed (fa) and apparent permeability coefficients (Papp) determined in Caco-2 cell model. Dotted line represents cutoff value for fa 50% and 85%.

Requirements:
Min 5 low, moderate and high permeability model drugs
Zero permeability marker
Min 3 cell assay replicates

Figure 2. (a) Correlation of human fraction absorbed (fa) and apparent permeability coefficients (Papp) determined in isolated rat jejunum tissue. Dotted line represents cutoff values for fa = 90% and Papp = 11 x 10^-6 cm/s. (b) Correlation of rat Papp and human Peff values. Results are shown as mean values; refer to Table 1 for sample size.
Validation of in-house Caco-2 (and rat intestine model)

Integrity/vitality criteria

**Caco-2**
- TEER @21 day of growth
- TEER start of exp
- TEER end of exp
- Lucifer yellow

**Rat intestine**
- TEER start of exp
- TEER end of exp
- PD start of exp
- PD end of exp
- PD after addition of glucose
- FITC dextran

TEER – transepithelial electrical resistance
PD – potential difference

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Demonstrating suitability of the Caco-2 cell model for BCS-based biowaiver according to the recent FDA and ICH harmonised guidelines, *Journal of Pharmacy and Pharmacology*, 2019.
Validation of in-house Caco-2 (and rat intestine model)

Gene expression

Gene expression for key absorptive and secretory transporters known.


Demonstrating suitability of the Caco-2 cell model for BCS-based biowaiver according to the recent FDA and ICH harmonised guidelines, *Journal of Pharmacy and Pharmacology*, 2019.
Validation of in-house Caco-2 (and rat intestine model)

P-glycoprotein expressed & functional. Inter-subject variability in ER on rat determined to be at least 2.
Case 1: Levetiracetam

Literature data
- Highly soluble
- Highly permeable
- Not a narrow therapeutic window drug
- In 2015, 282 levetiracetam IR products available

<table>
<thead>
<tr>
<th>Aim of the Study</th>
<th>Observed Effect</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of absorption</td>
<td>Rapid absorption, $T_{\text{max}} = 1.3$ h, 95% recovery in urine</td>
<td>Absorption is almost complete (&gt;95%)</td>
</tr>
<tr>
<td>Food Interaction</td>
<td>$T_{\text{max}}$ delayed, $C_{\text{max}}$ unaffected</td>
<td>Levetiracetam can be ingested with or without meals</td>
</tr>
<tr>
<td>Protein binding</td>
<td>$C_{\text{max}}$ and AUC are dose proportional in doses up to 5000 mg</td>
<td>Protein binding displacement interactions with other highly protein bound drugs is unlikely to occur</td>
</tr>
<tr>
<td>Distribution</td>
<td>$V_d = 0.5$–0.7 L/kg</td>
<td>Value close to total body water, that is, rapidly distributes into tissue with concentration approximating the one in blood.</td>
</tr>
<tr>
<td>Interactions with active transport/elimination</td>
<td>Non-hepatic, in blood</td>
<td>Absorption/elimination is most probably not modulated via active transporters.</td>
</tr>
<tr>
<td>Metabolism</td>
<td></td>
<td>Unlikely to produce clinically relevant interactions through induction/inhibition of CYP reactions</td>
</tr>
<tr>
<td>Plasma elimination $t_{1/2}$</td>
<td>6–8 h (healthy adults)</td>
<td>Dosage adjustments are not necessary when patients are co-medicated with other AEDs; adjustment needed in children and elderly population.</td>
</tr>
<tr>
<td></td>
<td>6–8 h (adults with epilepsy)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10–11 h (elderly) 5–7 h</td>
<td></td>
</tr>
</tbody>
</table>

Highest strength: 1000 mg
Max single dose: 3000 mg
Case 1: Levetiracetam

Solubility

High solubility in pH range 1.2 – 7.5 (0.72-0.74 g/ml) (shake-flask method).

Permeability

<table>
<thead>
<tr>
<th>Permeability Method and used Media</th>
<th>Compound</th>
<th>pH Donor/Acceptor</th>
<th>Permeability (×10⁻⁶ cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated rat intestine S to M</td>
<td>Levetiracetam</td>
<td>7.4/7.4</td>
<td>25.8 ± 5.2</td>
</tr>
<tr>
<td>(Ringer’s buffer solution)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated rat intestine M to S</td>
<td>Levetiracetam</td>
<td>7.4/7.4</td>
<td>30.6 ± 1.5</td>
</tr>
<tr>
<td>(Ringer’s buffer solution)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atenolol (low permeability marker)</td>
<td>7.4/7.4</td>
<td></td>
<td>8.9 ± 1.4</td>
</tr>
<tr>
<td>Metoprolol (high permeability marker)</td>
<td>7.4/7.4</td>
<td></td>
<td>22.0 ± 1.4</td>
</tr>
</tbody>
</table>

Dissolution

Very fast drug release: > 85% @ 15 min

Suitability of Isolated Rat Jejunum Model for Demonstration of Complete Absorption in Humans for BCS-Based Biowaiver Request, *Journal of Pharmaceutical Sciences*, 2012

Case 1: Levetiracetam

Published bioequivalence studies:

<table>
<thead>
<tr>
<th>Population</th>
<th>Dosage</th>
<th>Pharmacokinetic Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC&lt;sub&gt;t&lt;/sub&gt; (µg h/mL)</td>
</tr>
<tr>
<td>Healthy adults (N=24), fed conditions</td>
<td>500 mg tablets vs 000 mg capsules</td>
<td>15.74 ± 3.93 vs 15.33 ± 4.49</td>
</tr>
<tr>
<td>Healthy adults (N=22), fasting conditions</td>
<td>750 mg tablets test vs reference</td>
<td>19.64 ± 3.65 vs 20.05 ± 4.13</td>
</tr>
<tr>
<td>Healthy adults (N=21), fasting conditions</td>
<td>1000 mg tablets test vs reference</td>
<td>23.50 ± 6.35 vs 24.93 ± 4.43</td>
</tr>
<tr>
<td>Healthy adults (N=29), fasting conditions</td>
<td>1500 mg oral tablets vs i.v. solution</td>
<td>31.51 ± 8.54 vs 57.60 ± 15.89</td>
</tr>
<tr>
<td>Healthy adults (N=24), fasting conditions</td>
<td>750 mg oral tablets vs 10% oral solution</td>
<td>20.30 ± 3.9 vs 21.10 ± 4.0</td>
</tr>
</tbody>
</table>

Possible effects of excipients unlikely (several published studies, 282 products with various compositions found BE)

In-house bioequivalence study:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ratio LSM&lt;sup&gt;b&lt;/sup&gt; (Test/Reference)</th>
<th>90% Confidence Intervals</th>
<th>Intrasubject CV&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;t&lt;/sub&gt;</td>
<td>100.07%</td>
<td>97.44%–102.78%</td>
<td>5.0%</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;max&lt;/sub&gt;</td>
<td>100.03%</td>
<td>97.38%–102.75%</td>
<td>5.0%</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>99.95%</td>
<td>93.61%–106.71%</td>
<td>12.2%</td>
</tr>
</tbody>
</table>

Low variability of PKs. Low intrasubject CV. Narrow CI.

Case 2: Desloratadine

- Antihistamine drug
- IR tablets (1 tbl of 5 mg/day)
- Linear pharmacokinetics 5 - 20 mg in healthy
- Pgp substrate (grapefruit no impact on $C_{\text{max}}$, AUC)
- Literature suggests BCS 1 (?)

<table>
<thead>
<tr>
<th>Solubility (mg/mL)</th>
<th>Dissolved in 250 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>39.7 &gt; 9 g</td>
</tr>
<tr>
<td>Water</td>
<td>0.1 25 mg</td>
</tr>
<tr>
<td>Phosphate pH 7.4</td>
<td>1.5 375 mg</td>
</tr>
</tbody>
</table>

Desloratadine

- 2% in urine
- 7% in feces

3-OH-desloratadine

- 87.1% (half in urine and half in feces)

F_{abs} > 90 % (?)

Inconclusive data, no literature

When is fecal fraction of metabolites formed?

Biopharmaceutical classification of desloratadine – not all drugs are classified the easy way, *Acta Pharm*, 70 (2020) 131–144
Case 2: Desloratadine

**Solubility**

<table>
<thead>
<tr>
<th>Media</th>
<th>Equilibrium solubility (mg mL(^{-1})) and RSD</th>
<th>Volume of media needed to dissolve 5 mg dose (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mol L(^{-1}) HCl</td>
<td>40.49 (0.4 %)</td>
<td>0.123</td>
</tr>
<tr>
<td>Acetate buffer pH 4.5</td>
<td>10.65 (1.4 %)</td>
<td>0.469</td>
</tr>
<tr>
<td>Sodium phosphate buffer pH 6.8</td>
<td>2.46 (1.2 %)</td>
<td>2.03</td>
</tr>
<tr>
<td>Water</td>
<td>0.92 (3.4 %)</td>
<td>5.43</td>
</tr>
</tbody>
</table>

**Dissolution**

**Intraluminal stability**

Recoveries:
- 102.6 % in SIF
- 99.6 % in SGF
- 101.1 % in SGF with pepsin

Biopharmaceutical classification of desloratadine – not all drugs are classified the easy way, *Acta Pharm*, 70 (2020) 131–144
Case 2: Desloratadine

PAMPA

<table>
<thead>
<tr>
<th>DONOR pH</th>
<th>ACCEPTOR pH</th>
<th>Average DESLORATADINE permeability (×10^-6 cm s^-1)</th>
<th>Average METOPROLOL permeability (×10^-6 cm s^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>7.4</td>
<td>1.13 (n = 8; RSD 32 %)</td>
<td>1.09 (n = 8; RSD 31 %)</td>
</tr>
<tr>
<td>6.8</td>
<td>7.4</td>
<td>7.61 (n = 8; RSD 17 %)</td>
<td>4.60 (n = 8; RSD 25 %)</td>
</tr>
<tr>
<td>7.4</td>
<td>7.4</td>
<td>16.6 (n = 8; RSD 9.7 %)</td>
<td>9.09 (n = 8; RSD 22 %)</td>
</tr>
</tbody>
</table>

Rat intestine – side-by-side diffusion chambers

Unexposed standard solution in glassware (*), room temperature
- 100 %
- 99.1 %
Carbonated standard solution in glassware, 37 °C
- 100.4 %
- 101.3 %
Carbonated standard solution in side-by-side diffusion chambers, 37 °C
- 90.3 %
- 90.5 %
Carbonated standard solution incubated with intestinal rings, 37 °C
- 38.9 %
- 34.0 %

1 h 2 h

Desloratadine Metoprolol
1338 706 4.9 × 10^-7 9.5 × 10^-6 9.4 × 10^-6
1202 847 4.7 × 10^-7 16.7 × 10^-6

Caco-2

Direction | TEER start (Ω cm^-2) | TEER end (Ω cm^-2) | Lucifer Yellow (cm/s) | Desloratadine (cm/s) | Metoprolol (cm/s) |
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AP→BL</td>
<td>1338</td>
<td>706</td>
<td>4.9 × 10^-7</td>
<td>9.5 × 10^-6</td>
<td>9.4 × 10^-6</td>
</tr>
<tr>
<td>BL→AP</td>
<td>1202</td>
<td>847</td>
<td>4.7 × 10^-7</td>
<td>16.7 × 10^-6</td>
<td></td>
</tr>
</tbody>
</table>

Integrity and vitality
ER = 1.7
High permeability
Less biological material available for tissue binding.
Recoveries > 80%.

Permeability

High permeability
Not valid for agencies

Too low recoveries to determine \( P_{\text{APP}} \) (LC-MS no metabolites).
Incubation with intestinal rings show high tissue retention (50% recovered with tissue extraction).
10% plastic binding.
Case 3: Prodrug permeability classification

FDA guidance 2001; 2017:

**Prodrugs**
Permeability of prodrugs will generally depend on the mechanism and (anatomical) site of conversion to the drug substance. When the prodrug-to-drug (i.e., active moiety) conversion is shown to occur predominantly after intestinal membrane permeation, the permeability of the prodrug should be measured. When this conversion occurs prior to intestinal permeation, the permeability of the drug should be determined. Dissolution and pH-solubility data on both prodrug and drug can be relevant. Sponsors may wish to consult with appropriate review staff before applying the BCS approach to IR products containing prodrugs.

FDA guidance 2021:

Pro-drugs may be considered for a BCS-based biowaiver when absorbed as the pro-drug.

Should drug permeability in prodrug cases again be included in the guidelines?
Case 3: Prodrug permeability classification

Aim #1

To evaluate the rate of hydrolysis of a prodrug at 37°C when in the contact with the rat intestine (and its brush-border enzymes) at donor side (mucosal side) and after permeability (acceptor, serosal side) (Easy Mount diffusion (Ussing) chambers).

Experiments

Prodrug was added to the donor compartments. The samples were withdrawn from donor and acceptor compartments at predetermined time intervals.
Case 3: Prodrug permeability classification

RESULTS #1

- Prodrug is rapidly hydrolyzed at donor (mucosal) side by intestinal brush border enzymes, even prior drug absorption.

- The major compound detected in acceptor is drug, the hydrolysis most probably occurs also at acceptor side.

- It remains unclear what is the ratio between both forms at absorption/permeation.

- In the guidelines it is clear that prodrugs may be considered for a BCS-based biowaiver when absorbed as the pro-drug. However, in vivo very seldom only a prodrug is absorbed.

Literature data: The main site of prodrug-drug conversion is believed to be blood.
Case 3: Prodrug permeability classification

Aim #2
To evaluate the hydrolysis of Impurity 1 (pro-prodrug?) before and after permeation.

RESULTS #2

- Impurity 1 is formed during prodrug synthesis (process-related impurity).
  - It is hydrolyzed at donor (mucosal) side by intestinal brush border enzymes prior drug absorption.
  - This was confirmed with commercially available enzymes esterase and pancreatic lipase.
  - Due to presence of esterases in GIT and lipases excreted in duodenal fluid it can be concluded that Impurity 1 would hydrolyse rapidly to drug in human GIT.

Literature data:
Approx. 1,000 U of lipases per ml of duodenal fluid of adults. Between 25,000 and 40,000 U is required to digest a typical meal.
Conclusions

Permeability studies from generic perspective for BCS biowaiver support:

- BCS biowaiver approach is well accepted by the generic companies, because it can facilitate approval for highly soluble BCS1 and BCS3 drugs.

- Guidance could again allow complementary systems for drug permeability evaluation, such as rat intestine and other appropriate cell lines, to capture more complex absorption features (such as metabolism).

- BCS biowaiver approach could be applied to IR products containing prodrugs that convert to drugs also in the lumen or during permeation.
Thank you