Excipient effects:
In vitro and in vivo

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References

• BCS Class 3 excipient study

• Reply to “On the effect of common excipients on the oral absorption of class 3 drugs”
Topics

• In vitro
• In vivo BCS Class 3 excipient study
Biowaivers and BCS

• Biowaiver – waiver of need to demonstrate in vivo BE based on in vitro BE

• Apply biowaivers to less risky drugs, but which are those?!?

• Biopharmaceutics Classification System (BCS)
  – Based on solubility and intestinal permeability
  – Class 1 = high solubility and high permeability
  – Class 3 = high solubility and low permeability
    • Class 3 biowaivers: Excipients should not modulate drug absorption

Permeability/Absorption Systems

• Human
  – In vivo BA study
  – Perfusion, scintigraphy

• Animal
  – In vivo: intestinal ports, scintigraphy
  – In situ: perfusion
  – In vitro: Ussing chamber (tissue flap)

• Cell Culture
Caco-2 Cell Monolayers as Model for Intestinal Permeability

• Monolayer of human colon adenocarcinoma cells
• Typical advantages of in vitro (e.g. control, reduced variability, throughput)
• Typical disadvantages of in vitro (e.g. not in vivo!)
• Morphologically and biochemically similar to in vivo small intestinal epithelium
  – Polarized, columnar cells
  – Tight junctions
  – Microvilli
  – Polarized distribution of brush border enzymes
  – Form domes common to normal, transporting epithelium
  – Active transport systems of the small intestine
Excipient Considerations

• Excipient consideration
  – “Normal range”
  – Amounts exceeding “normal range” should be reviewed
  – FDA Inactive Ingredients Database
Fig. 2. Correlation between % dose absorbed *in vivo* in humans versus $P_{app}$ across Caco-2 cell monolayers treated with 10 μM CP-Y ($R = 0.95$, $n = 35$).
Identification of Active Drug Efflux

• Directional permeability studies in an efflux expressing cell model (e.g. Caco-2 expressing P-gp)
## Tween 80

<table>
<thead>
<tr>
<th>Drug</th>
<th>Papp (SEM) x $10^6$ [cm/sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Atenolol</td>
<td>0.592 (0.041)</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>0.405 (0.031)</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>1.26 (0.05)</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>0.650 (0.007)</td>
</tr>
<tr>
<td>Furosemide</td>
<td>0.466 (0.029)</td>
</tr>
<tr>
<td>HCTZ</td>
<td>0.710 (0.063)</td>
</tr>
</tbody>
</table>

* $p < 0.05$
## Effect of GF918 in MDCK-MDR1 Cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Direction</th>
<th>Dexloxiclumide $P \times 10^6$ (cm/sec)</th>
<th>B/A ratio of Dexloxiclumide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexloxiclumide alone</td>
<td>AP-BL</td>
<td>1.21 ($\pm 0.19$)</td>
<td>9.35 ($\pm 0.73$)</td>
</tr>
<tr>
<td></td>
<td>BL-AP</td>
<td>11.3 ($\pm 2.6$)</td>
<td></td>
</tr>
<tr>
<td>Dexloxiclumide plus GF 120918</td>
<td>AP-BL</td>
<td>2.11 ($\pm 0.24$)</td>
<td>1.03 ($\pm 0.03$)</td>
</tr>
<tr>
<td></td>
<td>BL-AP</td>
<td>2.18 ($\pm 0.27$)</td>
<td></td>
</tr>
</tbody>
</table>
**Excipient Effect on Ranitidine Permeability In Vitro**

<table>
<thead>
<tr>
<th>Croscarmellose Sodium concentration (mg/ml)</th>
<th>Caco-2 Papp x $10^6$ (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.425 (± 0.058)</td>
</tr>
<tr>
<td>0.001</td>
<td>0.650 (± 0.032)</td>
</tr>
<tr>
<td>0.01</td>
<td>0.808 (± 0.085)</td>
</tr>
<tr>
<td>0.06</td>
<td>11.2 (± 0.1)</td>
</tr>
</tbody>
</table>

- Increase ranitidine in vitro permeability via TJ dilation. Hence, in vitro test more sensitive than in vivo, where products were bioequivalent.
Topics

• In vitro
• In vivo BCS Class 3 excipient study
Drug Product Quality

Multiple Manufacturers → ANDA Approval → SUPAC Formulation(s) → Supplements → NDA Approval → New Drug Application

SUPAC Formulation(s) → Scale-up and post-approval changes

Further Development Formulation(s) → Safety and Efficacy

Clinical Trial Formulation(s) → Pharmacokinetics (PK)

Development Formulation(s)
Excipient Effects

• US FDA and EMA allow biowaivers of BCS class 3 drugs:
  – For excipients that are not known to affect bioavailability, BCS class 3 biowaivers require that excipients be qualitatively the same and quantitatively very similar

• BCS Class 3 drugs: site-dependent absorption properties
Study 1

- Cimetidine and acyclovir – BCS class 3 drugs
- 14 common excipients
- Three capsule formulations for each drug
- In vivo evaluation (2 capsules as single dose)
  - Fasted, single-dose, four-way crossover bioequivalence study (n=24) in healthy human volunteers
- Oral liquid used as reference product
- Average BE analysis to determine impact of excipients
Study 1

Two 4 way crossover BE study in healthy subjects

Study 1A

Cimetidine
BCS Class III

3 Test capsules: 3 excipients in each capsule

Reference: commercial oral Solution

Study 1B

Acyclovir
BCS Class III

3 Test capsules: 3 excipients in each capsule

Reference: commercial oral suspension
Top 20 excipients in BCS Class 3 drugs

- Magnesium Stearate
- Microcrystalline Cellulose
- Lactose
- Starch
- Sodium Starch Glycolate
- Silicon Dioxide
- Povidone
- Sodium Lauryl Sulfate
- Croscarmellose Sodium
- Stearic Acid

- Pregelatinized Starch
- Hydroxypropylmethyl Cellulose
- Opadry
- Crospovidone
- Talc
- Calcium Phosphate
- Citric Acid
- Sucrose
- Methyl Cellulose
- Titanium Dioxide
Test capsule formulations with 100mg cimetidine per capsule

<table>
<thead>
<tr>
<th>formulation</th>
<th>Excipient 1</th>
<th>Excipient 2</th>
<th>Excipient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CimTest-1</td>
<td>Microcrystalline Cellulose (300mg)</td>
<td>Hydroxypropyl-methyl Cellulose (45mg)</td>
<td>Sodium Lauryl Sulfate (25mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CimTest-2</td>
<td>Corn Starch (450mg)</td>
<td>Sodium Starch Glycolate (100mg)</td>
<td>Colloidal Silicon Dioxide (20mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CimTest-3</td>
<td>Dibasic Calcium Phosphate (300mg)</td>
<td>Sodium Lauryl Sulfate (25mg)</td>
<td>Crospovidone (50mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Passed QC testing, although not all very rapidly dissolving (although at least rapidly dissolving).
Test capsule formulations with 100mg acyclovir per capsule

<table>
<thead>
<tr>
<th>formulation</th>
<th>Excipient 1</th>
<th>Excipient 2</th>
<th>Excipient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcyTest-1</td>
<td>Microcrystalline Cellulose (300mg)</td>
<td>Hydroxypropyl-methyl Cellulose (45mg)</td>
<td>Sodium Lauryl Sulfate (25mg)</td>
</tr>
<tr>
<td>AcyTest-2</td>
<td>Lactose (450mg)</td>
<td>Povidone (35mg)</td>
<td>Stearic Acid (40mg)</td>
</tr>
<tr>
<td>AcyTest-3</td>
<td>Pregelatinized Starch (100mg)</td>
<td>Croscarmellose Sodium (60mg)</td>
<td>Magnesium Stearate (40mg)</td>
</tr>
</tbody>
</table>

Passed QC testing, although all were (only) rapidly dissolving.
Cimetidine mean profiles
Cimetidine BE analysis

<table>
<thead>
<tr>
<th>Formulation (vs CimTest-2)</th>
<th>Cmax point estimate</th>
<th>Cmax 90% CI</th>
<th>AUCt point estimate</th>
<th>AUCt 90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CimTest-1</td>
<td>90.6</td>
<td>81.0-101.3</td>
<td>90.9</td>
<td>84.9-97.2</td>
</tr>
<tr>
<td>CimTest-3</td>
<td>101.5</td>
<td>90.8-113.4</td>
<td>95.0</td>
<td>88.8-101.6</td>
</tr>
<tr>
<td>Solution</td>
<td>75.2</td>
<td>67.3-84.1</td>
<td>81.1</td>
<td>75.8-86.8</td>
</tr>
</tbody>
</table>

HPMC: retards drug release?

Reference oral solution contains sorbitol: increase gastrointestinal transit time?
Fig. 4. Mean plasma concentrations of ranitidine in 24 healthy volunteers after administration of 150 mg ranitidine solution with addition of 0 (closed circle), 1.25 (triangle), 2.5 (square), and 5 Gm (diamond) of sorbitol.

Acyclovir mean profiles

Same formulation and same effect for CimTest-1
## Acyclovir BE analysis

<table>
<thead>
<tr>
<th>Formulation (vs suspension)</th>
<th>Cmax point estimate</th>
<th>Cmax 90% CI</th>
<th>AUCt point estimate</th>
<th>AUCt 90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcyTest-1</td>
<td>82.7</td>
<td>72.1-94.9</td>
<td>91.7</td>
<td>80.4-104.7</td>
</tr>
<tr>
<td>AcyTest-2</td>
<td>102.9</td>
<td>89.7-118.1</td>
<td>97.4</td>
<td>85.3-111.2</td>
</tr>
<tr>
<td>AcyTest-3</td>
<td>87.1</td>
<td>75.9-99.9</td>
<td>87.6</td>
<td>76.7-99.9</td>
</tr>
</tbody>
</table>

HPMC: retards drug release?

High magnesium stearate with Turbula mixer cause over-lubrication?)
Study 1 Conclusions

• Fourteen excipients were evaluated
• Most excipients did not appear to impact BCS class 3 drug permeability
• CimTest-1 and AcyTest-1 exhibited lower exposure, probably due to HPMC impact on dissolution
• AcyTest-3 exhibited low exposure, probably due to magnesium stearate impact on dissolution
• The commercial cimetidine solution exhibited low exposure, perhaps due to level of sorbitol
Study 2

4 way cross over BE study: Cimetidine

CimTest-A: < 45mg HPMC

CimTest-B: < 40mg Mag Stearate

Commercial Cimetidine oral solution

Reference Solution: Oral solution without sorbitol
In Vitro Dissolution

- All capsules were very rapidly dissolving in media of pH 1.2, 4.5, and 6.8.
- CimTest-A contained 20mg HPMC (plus two others)
- CimTest-B contained 20mg magnesium stearate (plus two others)
Mean Cimetidine Profiles
## Cimetidine Average BE Results

<table>
<thead>
<tr>
<th>Formulation (vs reference)</th>
<th>$C_{\text{max}}$ point estimate</th>
<th>$C_{\text{max}}$ 90% CI</th>
<th>AUC$_{0-t}$ point estimate</th>
<th>AUC$_{0-t}$ 90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CimTest-A</td>
<td>122.1</td>
<td>109.4–136.2</td>
<td>112.2</td>
<td>104.4–120.6</td>
</tr>
<tr>
<td>CimTest-B</td>
<td>105.0</td>
<td>94.1–117.2</td>
<td>105.2</td>
<td>97.9–113.0</td>
</tr>
<tr>
<td>Commercial solution</td>
<td>86.9</td>
<td>77.9–97.0</td>
<td>100.2</td>
<td>93.2–107.7</td>
</tr>
<tr>
<td>Excipient</td>
<td>Recommended maximum allowable amount for a class 3 biowaiver (mg)</td>
<td>Maximum excipient amount studied here (mg)</td>
<td>Typical excipient amount (when present) in an IR tablet or capsule with a total weight of 300mg</td>
<td>Maximum amount (mg) in Inactive Ingredient Database</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------------------------------------------------</td>
<td>---------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Microcrystalline Cellulose</td>
<td>Qualitatively same and quantitatively very similar</td>
<td>600</td>
<td>100mg (20%-90%)</td>
<td>1385.3</td>
</tr>
<tr>
<td>Hydroxypropyl Methyl Cellulose</td>
<td>Qualitatively same and quantitatively very similar</td>
<td>40</td>
<td>10mg (2%-5%)</td>
<td>444.4</td>
</tr>
<tr>
<td>Sodium Lauryl Sulfate</td>
<td></td>
<td>50</td>
<td>4.5mg (0.5%-2.5%)</td>
<td>51.69</td>
</tr>
<tr>
<td>Corn Starch</td>
<td></td>
<td>900</td>
<td>150mg (25%-75%)</td>
<td>1135</td>
</tr>
<tr>
<td>Sodium Starch Glycolate</td>
<td></td>
<td>200</td>
<td>12mg (4%)</td>
<td>876</td>
</tr>
<tr>
<td>Colloidal Silicon Dioxide</td>
<td></td>
<td>40</td>
<td>1.5mg (0.1%-1%)</td>
<td>100</td>
</tr>
<tr>
<td>Dibasic Calcium Phosphate</td>
<td></td>
<td>600</td>
<td>150mg (25%-75%)</td>
<td>635.5</td>
</tr>
<tr>
<td>Crospovidone</td>
<td></td>
<td>100</td>
<td>10mg (2%-5%)</td>
<td>340</td>
</tr>
<tr>
<td>Lactose</td>
<td></td>
<td>900</td>
<td>240mg (80%)</td>
<td>1020</td>
</tr>
<tr>
<td>Povidone</td>
<td></td>
<td>70</td>
<td>7.5mg (0.5%-5%)</td>
<td>240</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td></td>
<td>80</td>
<td>6mg (1%-3%)</td>
<td>72</td>
</tr>
<tr>
<td>Pregelatinized Starch</td>
<td></td>
<td>200</td>
<td>150mg (5%-75%)</td>
<td>435.8</td>
</tr>
<tr>
<td>Croscarmellose Sodium</td>
<td></td>
<td>120</td>
<td>37.5mg (0.5%-25%)</td>
<td>180</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td></td>
<td>40</td>
<td>7.5mg (0.25% to 5%)</td>
<td>400.74</td>
</tr>
</tbody>
</table>
Conclusions

• Fourteen commonly used excipients in IR solid oral dosage forms were evaluated
• 12 out of 14 were found to be non-problematic: should be no more than quantities studied
• HPMC and microcrystalline cellulose: should be qualitatively the same and quantitatively similar to reference product
Topics

• In vitro
• In vivo BCS Class 3 excipient study