Biorelevant media for in vitro permeability assessment of phosphate ester prodrugs: a case study with fosamprenavir

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GPEN, Kansas, October 25th 2006
Introduction

intraluminal drug & formulation behavior
Introduction

intraluminal drug & formulation behavior

Intraluminal conditions in function of time after oral drug intake?

- pH
- bile salts
- phospholipids
- drug concentration
- excipient concentration
- influence of food
...

Diagram with various labeled components (likely representing different parts of the digestive tract and the drug formulation process).
Introduction

intraluminal drug & formulation behavior

Sampling of human gastro-intestinal fluids

- healthy volunteers
- double lumen catheter(s)
- blank fluid
- after intake of oral dosage form
- fasted vs fed
Introduction

*intraluminal drug & formulation behavior*

Sampling of human gastro-intestinal fluids

- characterization: pH, bile salts, phospholipids, drug, excipient…
  - intraluminal conditions (after oral drug intake)
    - descriptive
    - relation to pharmacokinetics
    - working mechanisms of formulations
    - …

- integration in in vitro studies (dissolution / solubility / stability / permeability)
  - influence of real intraluminal conditions on drug absorption
  - biorelevance of model systems: aqueous buffers vs intraluminal conditions
Introduction

amprenavir / fosamprenavir

Amprenavir:
- HIV protease inhibitor
- poorly water-soluble (0.08 mM in H$_2$O, pH 7, 37 °C)
- substrate of the efflux carrier P-gp

Standard formulation (Agenerase®):
- high pill burden!
- soft gelatin capsules
- amprenavir 150 mg (single dose: 8 capsules, amprenavir 1200 mg)
- solubilizing excipient TPGS

![Chemical structure of Amprenavir and TPGS](image-url)
Introduction

amprenavir / fosamprenavir

Fosamprenavir
water-soluble

→ Telzir®:
  2 tablets (1400 mg APV)

Amprenavir
poorly water-soluble

→ Agenerase®:
  8 capsules (1200 mg APV)
Introduction

*amprenavir / fosamprenavir*

Prodrug with increased solubility compared to parent drug

→ enhanced intestinal absorption of parent drug!

⇒ What happens in the gastro-intestinal tract?

- in vivo study
- in vitro study
Purpose

To characterize the in vitro behavior of fosamprenavir in the Caco-2 model system using different media:

- transport medium (aqueous buffer)
- human intestinal fluids
- “biorelevant” media: FaSSIF (+ taurocholate/phospholipids)
Methods

Stability of fosamprenavir?

transport medium (MES-buffered HBSS, pH 6.5) “biorelevant” media
human intestinal fluids (HIF)

Sampling of HIF
- 3 volunteers
- duodenum
- fasted state
- in function of time
- samples pooled per volunteer
- pH / inorganic phosphate

Sampling in function of time
- (filtration)
- analysis of amprenavir and fosamprenavir
  (HPLC + fluorescence detection)
Results

Fosamprenavir in transport medium / Caco-2

Stability of fosamprenavir upon incubation in transport medium?

Transport medium: MES-buffered HBSS pH 6.5
Results

Fosamprenavir in transport medium / Caco-2

Incubation at the apical side of Caco-2 monolayers (60 min, 37°C)

⇒ Conversion to amprenavir: concentration-dependent
  pH-dependent

⇒ Ca. 8% of the amprenavir formed is transported across the cell monolayer
Results

IAP: intestinal alkaline phosphatase
Results

*fosamprenavir in HIF / Caco-2*

Stability of fosamprenavir upon incubation in HIF?

HIF (human intestinal fluid): 3 volunteers, fasted, duodenum
Results

**fosamprenavir in HIF**

Stability of fosamprenavir (10 µM) upon incubation in HIF (37°C)

![Graph showing the stability of fosamprenavir over time for Subjects A, B, and C.](image)

⇒ Phosphatase activity in HIF, depending on subject.
Results

*fosamprenavir in HIF / Caco-2*

Incubation at the apical side of Caco-2 monolayers
(fosamprenavir 10 µM, 60 min, 37°C)

⇒ Phosphatase activity of Caco + HIF
High dose of fosamprenavir $\rightarrow$ amprenavir??

incubation of fosamprenavir 500 µM in HIF

Results

supersaturation in HIF
High dose of fosamprenavir $\rightarrow$ amprenavir??

incubation of fosamprenavir 500 µM in HIF

$\Rightarrow$ create and maintain supersaturation of amprenavir in HIF
Results

supersaturation and flux

Supersaturation $\Rightarrow C_0 \uparrow \Rightarrow$ flux?

incubation of amprenavir/fosamprenavir in HIF at Caco-2 monolayers

$\rightarrow$ transport of amprenavir in function of time?

![Graph showing Amprenavir transport over time]

APV “1 mM” = suspension
(in solution: 150 µM)
Results

supersaturation and flux

Supersaturation $\Rightarrow C_0 \uparrow \Rightarrow$ flux?

incubation of amprenavir/fosamprenavir in HIF at Caco-2 monolayers

$\rightarrow$ transport of amprenavir in function of time?

![Graph showing Amprenavir transport over time.]

FPV 1 mM = solution

APV “1 mM” = suspension
(in solution: 150 µM)
Results

supersaturation and flux

Supersaturation $\Rightarrow C_0 \uparrow \Rightarrow$ flux?

incubation of amprenavir/fosamprenavir in HIF at Caco-2 monolayers

$\rightarrow$ transport of amprenavir in function of time?

APV supersaturated solution

FPV 1 mM = solution

APV “1 mM” = suspension
(in solution: 150 µM)
Results

*fosamprenavir in FaSSIF / Caco-2*

Stability of fosamprenavir upon incubation in FaSSIF?

FaSSIF (Fasted State Simulated Intestinal Fluid): phosphate buffer pH 6.5

- taurocholate 3 mM
- lecithin 0.75 mM

FaSSIF

poorly water-soluble drugs

![Diagram of FaSSIF setup]
Results

*fosamprenavir in FaSSIF / Caco-2*

Incubation at the apical side of Caco-2 monolayers
(fosamprenavir 10 µM, 60 min, 37°C)

⇒ Almost no dephosphorylation of fosamprenavir using FaSSIF as medium!
→ not biorelevant!
Results


diinorganic phosphate

Incubation at the apical side of Caco-2 monolayers

(fosamprenavir 10 µM, 60 min, 37°C)

TM with different concentrations of inorganic phosphate (P_i)

⇒ inhibition of intestinal alkaline phosphatase by inorganic phosphate
## Results

**Inorganic phosphate**

Intraluminal phosphate concentrations?

<table>
<thead>
<tr>
<th></th>
<th>Subject A</th>
<th>Subject B</th>
<th>Subject C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$P_i$ (mM)</strong></td>
<td>0.0</td>
<td>0.5</td>
<td>1.0</td>
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<tr>
<td></td>
<td>1.5</td>
<td>2.0</td>
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</tr>
</tbody>
</table>

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*FaSSIF (28.7 mM) TM (0.8 mM)*
Results

**inorganic phosphate**

Intraluminal phosphate concentrations?

$$P_i \text{ (mM)}$$

- Subject A
- Subject B
- Subject C

$28.7 \text{ mM (FaSSIF)}$

⇒ Phosphate-buffered FaSSIF is not compatible with phosphate ester prodrugs.
Results

**inorganic phosphate**

Intraluminal phosphate concentrations?

⇒ Phosphate-buffered FaSSIF is not compatible with phosphate ester prodrugs.
Results

*alternative for FaSSIF*

Incubation at the apical side of Caco-2 monolayers
(fosamprenavir 10 µM, 60 min, 37°C)

**TM** (MES-buffered HBSS)  
+ taurocholate (TC) 3 mM  
+ phospholipids (PL) 0.75 mM

![Bar chart](image-url)

- **Amprenavir in acceptor**
- **Amprenavir in donor**
Conclusion

- Illustration of intraluminal supersaturation of a poorly water-soluble drug from its soluble prodrug in real intestinal media.
- Dephosphorylation of fosamprenavir is inhibited by inorganic phosphate → biorelevant media!
- Ongoing: in vivo intraluminal behavior of fosamprenavir fasted vs fed
Acknowledgements

• Onderzoeksfonds K.U.Leuven

• FWO-Vlaanderen

• Center for Gastro-enterologic Research, UZ Leuven
  Rita Vos
  Jan Tack

• Laboratory for Pharmacotechnology and Biopharmacy, K.U.Leuven
  Patrick Augustijns