Complex in vitro models to study oral application and exposure

Berlin, 8. Mai 2012
eva-maria.collnot@helmholtz-hzi.de
Helmholtz Institute for Pharmaceutical Research Saarland
Department of Drug Delivery (DDEL)
Inflammatory bowel disease

- A group chronic or recurrent inflammatory conditions of the colon and small intestine (Crohn’s Disease and Ulcerative Colitis)
- Symptoms: diarrhea, weight loss, pain
- Treatment: induction and maintenance of remission using immunosuppressents, glucocorticoids, monoclonal antibodies (anti TNF-α)
State of the art: animal models in drug/formulation development for IBD treatment

Rodent colitis models
- Transgenic
- Chemically induced, e.g. TNBS, DSS

Symptoms: Diarrhea, rectal bleeding, weight loss, pain, colon perforation, sepsis, death

Evaluation of treatment: scoring system, histological stainings, weight and length of colon

Issues: unethical, differences in species and pathogenesis

Arita M et al. PNAS 2005;102:7671-7676
The work horse: the Caco-2 model

Caco-2 monolayer

Intestinal mucosa
Adding complexity: immune cells
In vitro model of the inflamed intestinal mucosa

- Co-culture of Caco-2 intestinal epithelial cells with blood derived dendritric cells and macrophages
- Stimulation of inflammation by addition of lipopolysaccharides or pro-inflammatory cytokines (interleukin-1β) to the cell culture medium
- Reflects the relevant pathophysiological changes occurring in vivo
In vitro model of the inflamed intestinal mucosa
In vitro model of the inflamed intestinal mucosa: release of pro-inflammatory marker IL-8

In vitro model of the inflamed intestinal mucosa: changes in barrier properties

IL-1β stimulation

Tight junction protein ZO-1

Pathophysiological changes in the inflamed mucosa: Threat or potential?

Healthy mucosal barrier

Inflamed mucosal barrier

Collnot et al, J Control Release 2012, in press
Budesonide formulations for the treatment of IBD

Budesonide PLGA nanoparticles

- size ~220 nm, PDI: 0.08
- encapsulation rate: 67 µg/mg
- encapsulation efficiency: 46%

Liposomal budesonide

- size ~ 200 nm, PDI: 0.05
- encapsulation rate: 4.2 mg/ml
- encapsulation efficiency: 4.2%

Diluted or suspended in Caco-2 medium to a concentration of 100 µg/ml
Testing of anti-inflammatory formulations in the inflamed 3D model

Leonard et al, ALTEX, accepted
Testing of anti-inflammatory formulations in the inflamed 3D model

*Budesonide PLGA nanoparticles*  
*Liposomal budesonide*
Toxicity testing of engineered nanoparticles: InLiveTox
Toxicity testing of engineered nanoparticles: the candidates

<table>
<thead>
<tr>
<th>NP</th>
<th>Nominal</th>
<th>Water</th>
<th>Complete medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag, PVP capped, NM300</td>
<td>&lt; 20 nm</td>
<td>150 nm</td>
<td>120 nm</td>
</tr>
<tr>
<td>TiO₂ NM 101</td>
<td>7-10 nm</td>
<td>&gt;1000 nm</td>
<td>800 nm</td>
</tr>
<tr>
<td>Au, Phosphine capped</td>
<td>15 nm</td>
<td>20 nm</td>
<td>51 nm</td>
</tr>
<tr>
<td>Au, Phosphine capped</td>
<td>80 nm</td>
<td>88 nm</td>
<td>116 nm</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>55 nm</td>
<td>60 nm</td>
<td>55 nm</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>211 nm</td>
<td>230 nm</td>
<td>550 nm</td>
</tr>
</tbody>
</table>
Toxicity testing in the co-culture model: LDH release

Caco-2 monoculture EC50 = 82 µg/cm²
not inflamed triple culture EC50 = 364 µg/cm²
inflamed triple culture EC50 = 216 µg/cm²
What’s the reason for the differences observed?

Relative cell numbers in the mature 3D culture:

~$10^6$ Caco-2 cells vs. $10^4$ immune cells

→ LDH leakage and drop in metabolic activity is mainly associated with the Caco-2 cells
→ Reduced exposure of epithelial cells in the presence of immune cells due to preferential uptake by dendritic cells and macrophages?
Toxicity testing in the co-culture model: IL-8 production

![Graph showing IL-8 release vs Ag NP concentration for different cultures including baseline non-inflamed, baseline inflamed, baseline Caco-2, and Caco-2 non-inflamed triple culture with inflamed triple culture.]

- IL-8 release [pg/ml]
- Ag NP concentration [µg/cm²]
Toxicity testing in the co-culture model

- Nanoparticles
- Caco-2 cells
- Filttrer membrane
Toxicity testing in the co-culture model

Protective function of the immune cells in the 3D culture:
• preferential uptake of NM300 Ag NP
• reduced exposure of epithelial cells
• induction of immune answer in response to xenobiotic threats
Toxicity testing of engineered nanoparticles: InLiveTox
Dynamic cell culture and interconnected systems: InLiveTox
The next generation: The InLiveTox system
-Interconnected culture and systemic uptake-

Upper circuit, medium reservoir

Lower circuit, medium reservoir

ILT0: C3A

ILT0: HUVEC

ILT2: Caco-2

Bubble trap
It's all about support: silica membranes
It’s all about support: silica membranes
It’s all about support: improved transport properties
It’s all about support: automated TEER measurements
Summary

New tools for pharmakokinetic/toxikokinetik and mechanistic in vitro studies

- 3D co-culture model of the non-inflamed and inflamed intestinal mucosa
- Modular two flow system with automated TEER measurements
- Silica nitride based membranes with good cell growth properties and improved transport behaviour for macromolecules and nanoparticles
Acknowledgements

- HIPS/Saarland University
  Fransisca Leonard
  Julia Susewind
  Nadia Ucciferri
  Claus-Michael Lehr

- Utrecht University
  Bart Crielaard
  Twan Lammers
  Gert Storm

- Centre Suisse d’Electronique et de Microtechnique
  Sher Ahmed
  Melanie Favre
  Silvia Angeloni
  Martha Liley

- University of Pisa, Department of engineering
  Tommaso Sbrana
  Arti Ahluwalia

Thank you for your attention!