In vitro co-culture model of the inflamed intestinal mucosa

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Departement 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
In vitro test systems for oral bioavailability

Caco-2 monolayer

Intestinal mucosa
Adding complexity: immune cells
3D in vitro model of the inflamed intestinal mucosa

- Co-culture of Caco-2 intestinal epithelial cells with blood derived dendritic cells and macrophages

- Stimulation of inflammation by addition of lipopolysaccharides or pro-inflammatory cytokines (interleukin-1β) to the cell culture medium

- Should reflect the relevant pathophysiological changes occurring in vivo: release of pro-inflammatory markers (IL-8, TNF-α), re-organisation of tight junctional proteins, reduced barrier function, increased mucus production
Pathophysiological changes in the 3D model

*Infiltration of immunocompetent cells (macrophages + dendritic cells)*

Pathophysiological changes in the 3D model

*Upregulation and release of pro-inflammatory markers, e.g. IL-8 or TNF-α*

Pathophysiological changes in the 3D model

Changes in tight junctional organization (ZO-1) …

Pathophysiological changes in the 3D model

... and barrier function

*Leonard et al, Mol Pharm: 7(6), 2103-19 (2010)*
Pathophysiological changes in the 3D model

*Increased mucus production*

Pathophysiological changes in the 3D model

**Increased activity of immune cells**

Pathophysiological changes in the inflamed mucosa: Threat or potential?

Healthy mucosal barrier

Inflamed mucosal barrier

- Macrophage
- Intestinal epithelial cell
- Tight junctions
- Microparticle
- Nanoparticle
In vivo investigations in human IBD patients

Confocal laser endoscopy

Fluorescent PLGA nanoparticles

In vivo investigations in human IBD patients

In collaboration with C. Schmidt, C. Lautenschläger, A. Stallmach, University Hospital Jena

Accumulation of FA-PLGA microparticles in the rectal mucosa of human IBD patients

Moderately inflamed mucosa

Highly inflamed mucosa with flat ulcerations

Schmidt et al, Gut, submitted
Budesonide formulations for the treatment of IBD

In collaboration with B. Crielaard, T. Lammers, G. Storm, Utrecht University

Budesonide PLGA nanoparticles  Liposomal budesonide

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

- 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
Testing of anti-inflammatory formulations in the inflamed 3D model

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

Budesonide PLGA nanoparticles

Liposomal budesonide

Leonard et al, EJPB, submitted
Other applications of the 3D model of the inflamed intestinal mucosa: nanotoxicology

Interaction of the susceptible, inflamed intestinal barrier with (engineered) nanoparticles and other xenobiotics

Particle translocation and downstream signaling to endothelial cells and hepatocytes
Other applications of the 3D model of the inflamed intestinal mucosa: nanotoxicology

Significant change in response pattern compared to single culture:

Reduced epithelial damage

Increased inflammatory reaction
It’s only a matter of support: new directions for advanced intestinal cell models
It's only a matter of support: new directions for advanced intestinal cell models
Summary

- Successful establishment of a novel cell culture model simulating the intestinal mucosa in the state of inflammation

- Pathophysiologic changes reflected in the model: release of pro-inflammatory markers, activation of immune cells, decreased barrier function, re-organization of tight junctions, increased mucus production

- Applications of the model:
  - anti-inflammatory drug and formulation testing in pharmaceutical development
  - investigation of the interaction of (engineered) nanoparticles or other xenobiotics with the susceptible barrier

- Advantages over existing animal models: ethical aspect, no species differences, similar pathogenesis, mechanistical insight, cost and time reduction
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