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In vitro models to determine the pre-systemic metabolism of the human intestine following oral administration

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ABSTRACT

In the EU-funded project RISK-HUNT3R, our focus is to study external exposure to chemicals and drugs through three primary routes: the lung, skin, and intestine. To achieve this goal, we are developing new approach methodologies (NAM) that improve the reflection of human absorption barriers The more in vivo-like parameters generated in these models are then used to provide data to predictive toxicokinetic models of human exposure. The assessment of human oral exposure typically begins with measurements of the permeability of a given compound across monolayers of intestinal epithelial cells. Permeability provides a rough estimate of the fraction absorbed after oral administration. To enhance estimations, a measure of the fraction metabolized by enterocytes of the small intestinal epithelium is also required. Typically, Caco-2 cell model is utilized to measure permeability. A weakness of Caco-2 cells is their absence of major metabolizing enzymes found in the human intestine.

ENTEROCYTES AND ENTEROIDS

To determine intestinal metabolism of compounds of interest, we isolate primary human jejunal enterocytes that should retain all metabolizing enzyme activities. Human jejunal mucosa samples are obtained from patients undergoing gastric bypass surgery at Uppsala University Hospital to isolate enterocytes. We purify the enterocytes with a gentle, enzyme-free method. We are also cultivating small intestinal 3D organoids originating from human jejunal stem cells for permeability and metabolism analysis.
This enterocyte isolation produces a substantial amount of highly viable (> 90%) enterocytes, with 25-35% of these cells expressing caspase-8 and entering early apoptosis.

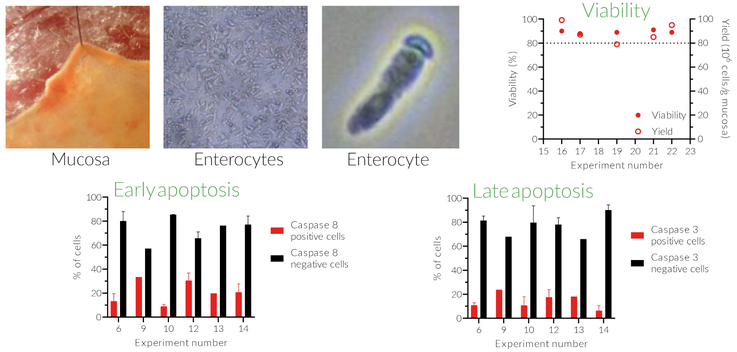

Figure 1: Enterocyte preparation from jejunal mucosa. Enterocytes are isolated with high viability, yield and limited apoptosis. Only a small fraction of the isolated enterocytes show apoptosis markers.

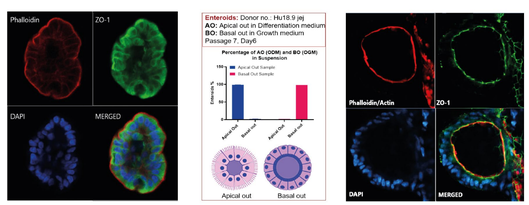

Fig. 2 Development of small intestinal 3D organoids for permeability and metabolism:
The enteroids are derived from human jejunal stem cells. They can be cultured in two configurations: apical out (left) and basal out (right). Efficiency of differentiation is shown in the middle panel. They were used for CYP enzyme activity and protein expression analysis.

ADME PROTEIN EXPRESSION

Enteroids show expression of many ADME relevant enzymes as determined by targeted proteomics.
- Expression levels are often, but not always close to the levels seen in tissue and enterocytes.
- In contrast Caco-2 cells show little to no expression of these enzymes.
- Enteroids express many intestinal transporters.

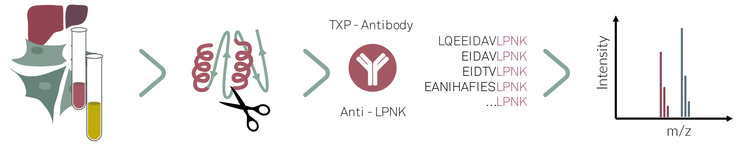

Figure 3: Workflow of immunoaffinity-MS. Proteins are tryptically digested and isotopically labeled standard peptides are added (I). Group-specific antibodies targeting C-terminal or common sequence motifs are employed in an automated immunoprecipitation workflow to enrich peptides derived from proteins of interest and standards (II). Finally unique peptides derived from the protein biomarkers are quantified via reference peptides and targeted nLC-mass spectrometry (III).

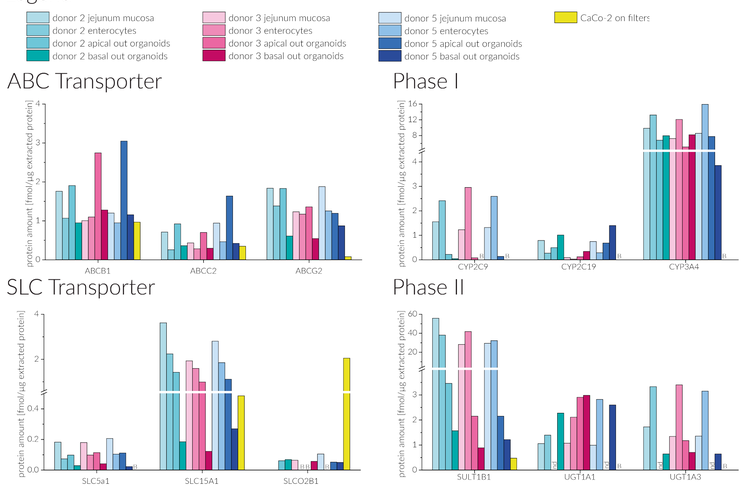

Figure 4: ADME protein expression. Protein expression was determined in jejunal tissue as well as in enterocytes and enteroids prepared from the same tissue and set in relation to Caco-2 cultures. The quantification of three ABC transporters, SLC transporters, phase I and phase II enzymes each is represented. CYPs and UGTs were below quantification limit in case of Caco-2 cells. (nd: not determined, B: below quantification limit)

CYP3A4 METABOLISM

Activity of several major intestinal CYPs (3A4, 2C9, 2D6) can be consistently detected and quantified in human primary enterocytes and enteroids.
• CYP3A4 activity data is shown in figure 5.
• As basolateral membranes are exposed in isolated enterocytes it is possible that intracellular exposure is higher than in intact epithelium.

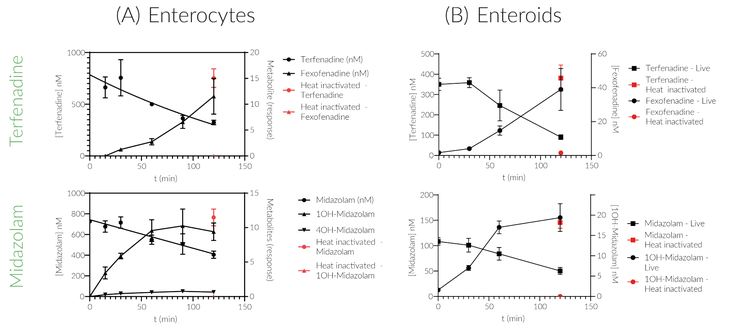

Figure 5: CYP3A4 metabolic activity in enterocytes and apical out enteroids. CYP3A4 activity can be detected as loss-of-parent (Terfenadine and Midazolam) and by metabolite formation (Fexofenadine and 1OH-Midazolam)

SUMMARY AND OUTLOOK

- Reproducible enterocyte isolation as well as enteroid formation and cultivation
- Enteroids can be cultured in two configurations: apical out or basal out
- CYP3A4 activity can be detected as loss-of-parent (Terfenadine and Midazolam) and by metabolite formation
- ADME protein expression in enterocytes and enteroids reflects mucosa tissue better than Caco-2 cultures

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