Advancing Drug Research with the InTESTine™ Platform: Ex Vivo Intestinal Explant Model for Peptides and Permeability Enhancers

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INTRODUCTION

Oral delivery of therapeutic peptides is the preferred route of administration but remains a significant challenge due to their low intestinal absorption. *In vitro* evaluation of permeation enhancers is complex, as current preclinical models often fail to properly reflect the complex physiology of the human gut, such as tight junction integrity and active mucus secretion, limiting their translational relevance.

AIM

Here we used the ex vivo tissue barrier model (InTESTine™) to evaluate the efficacy of two leading permeation enhancers, C10 and SNAC on the intestinal permeability of flux marker and clinical peptides. Additionally, we evaluate the effect of mucus on peptide permeability using fluorescently labelled peptides.

METHODS

- The InTESTine™ is a physiologically relevant intestinal ex vivo tissue model, creating a transwell set-up [1,2].

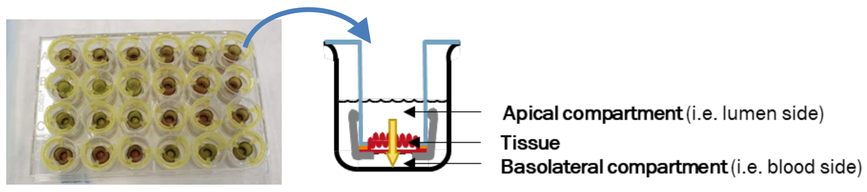

***Figure 1**. InTESTine™ intestinal ex vivo tissue explant model in a 24 well plate*

- Porcine intestinal tissue was applied to the 2-compartment InTESTine™ model
- Apical to basolateral permeability (apparent permeability (P_{app}) or % of dose) of flux markers FITC-dextran 4 kDa and 20 kDa, [^{3}H]-Mannitol, [^{14}C]-Caffeine, and peptides/immunoglobulins, Oxytocin, Insulin and IgG was calculated in the presence and absence of permeation enhancers sodium caprate (C10) and Salcaprozate sodium (SNAC).
- To study mucus adherence, intestinal explants were incubated with three GFP-labelled peptides, snap-frozen then visualized by confocal microscopy.
- Tissue viability was evaluated using LDH secretion into the apical and basolateral compartments.

RESULTS

The impact of mucus on the intestinal absorption of peptides

- Higher mucus adherence of GFP-labelled peptides resulted in lower permeability through the stomach and small intestinal barrier.

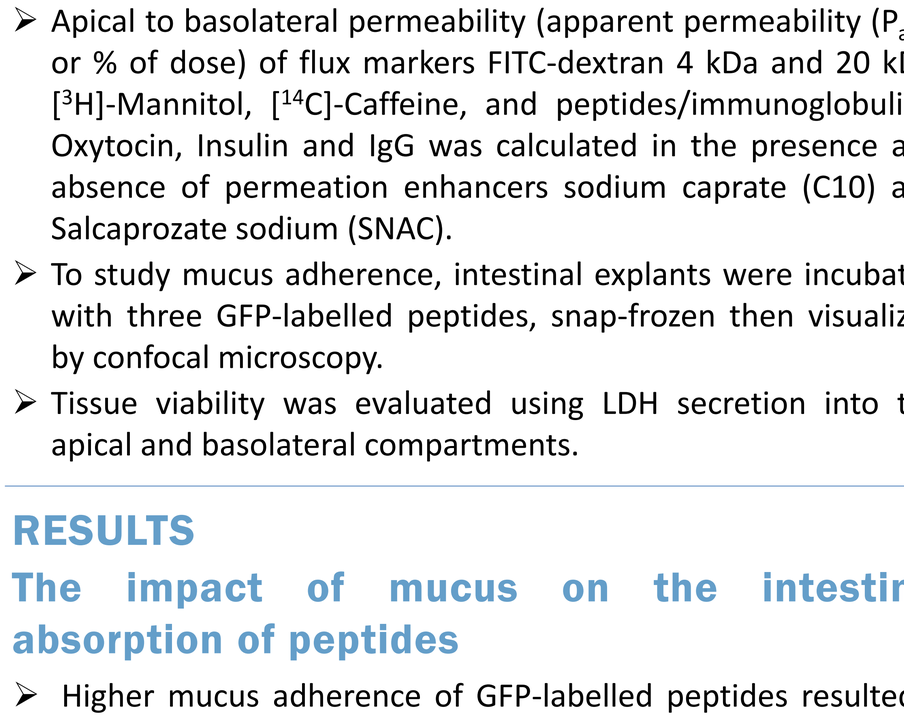

***Figure 2.** Porcine stomach and jejunum tissue explants in InTESTine™ were exposed to GFP labelled peptides. A-B) Percentage of dose in the apical and basolateral compartment up to 4h. C) Representative confocal microscopy images of the adherence of GFP-labelled peptides to porcine stomach tissue. Data: mean ± SD*

REFERENCES

[1] Stevens et al., Eur J Pharm Sci. (2019) Sep 1:137:104989.;
[2] Westerhout et al., Eur J Pharm Sci. (2014) Oct 15:63:167-77

RESULTS

Evaluation of permeation enhancers using flux markers

- Exposure of porcine jejunum tissue to escalating doses of C10 resulted in a dose-dependent increase in intestinal permeability of FD4, FD20 and Mannitol, particularly at earlier time points.
- Exposure of tissue to escalating doses of SNAC slightly increased intestinal permeability, particularly at later time points
- C10, but not SNAC, significantly increases intestinal tissue uptake of flux marker Mannitol in a dose-dependent manner
- Caffeine absorption was not affected upon co-incubation with C10 or SNAC (data not shown)

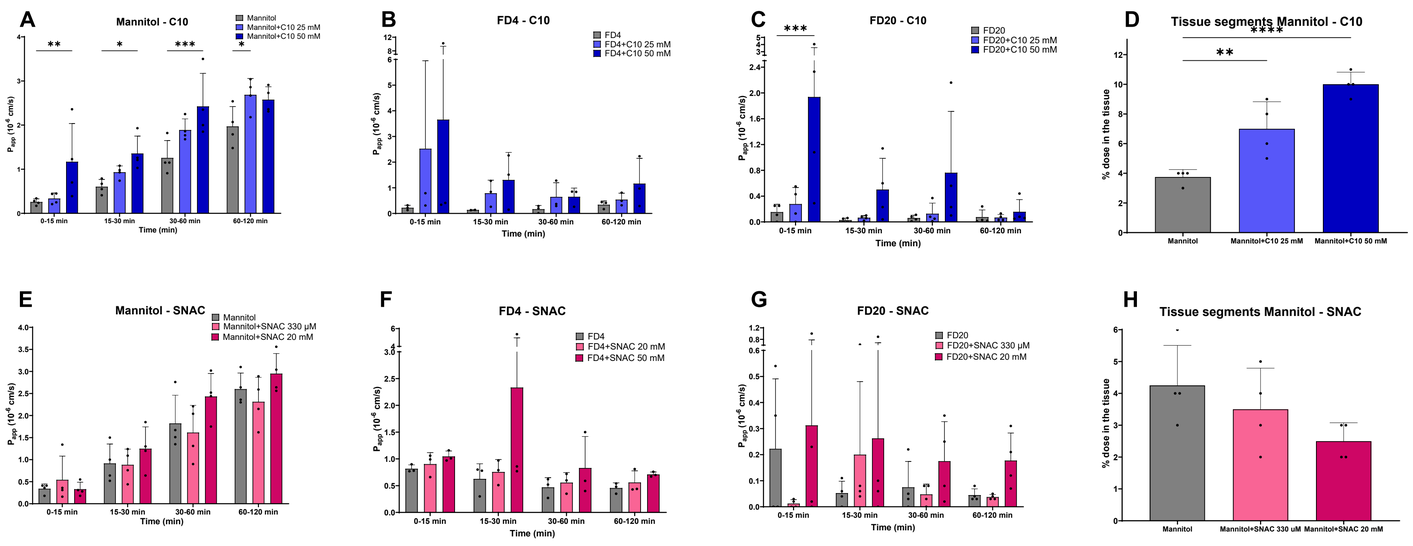

***Figure 3.** Porcine jejunum tissue explants in InTESTine™ were exposed to flux markers in the presence and absence of escalating doses of permeation enhancers C10 and SNAC (2 donors). A-C, E-F) The apparent permeability (P_{app}) of Mannitol, FD4 and FD20 were calculated at different time intervals. D, H) Percentage of Mannitol in the tissue the presence and absence of PEs. Data expressed as mean ± SD. *p<0.05 compared to control.*

RESULTS

Evaluation of permeation enhancer efficacy with clinical peptides

- C10 significantly increased the intestinal permeability of Oxytocin, Insulin, and IgG, especially at earlier time points. The effect diminished towards the end of the exposure period.
- SNAC enhanced the intestinal permeability of Oxytocin and Insulin, and to a lesser extent IgG, with a delayed onset compared to C10.
- C10 significantly increased intestinal tissue concentrations of Oxytocin and IgG, whilst Insulin was not detected in the tissue.
- C10 and SNAC did not affect tissue viability compared to the control.

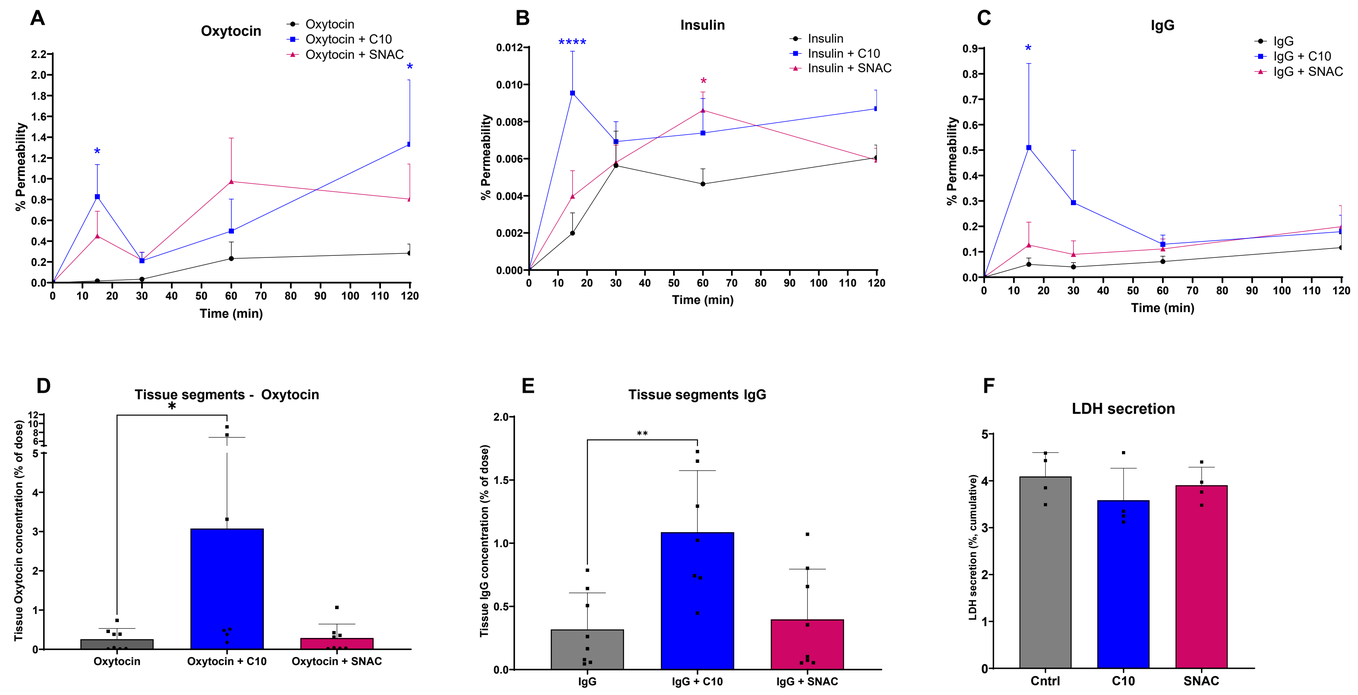

***Figure 4.** Porcine jejunum tissue explants in InTESTine™ were exposed to clinical peptides/immunoglobulins in the presence and absence of C10 (25 mM) and SNAC (330 µM). (2 donors). A-C) Percentage permeability of Oxytocin, Insulin and IgG through the intestinal barrier in the presence and absence of PEs. D-E) Percentage Oxytocin and IgG in the tissue compared to dose in the presence and absence of PEs. F) cumulative LDH secretion into the apical and basolateral compartment after 2h. Data expressed as mean ± SD. *p<0.05 compared to control.*

CONCLUSIONS AND FUTURE PERSPECTIVES

- We have successfully demonstrated the applicability of the ex vivo InTESTine™ model for screening permeation enhancers by quantifying macromolecule and peptide permeability through the physiologically relevant intestinal barrier.
- Using ex vivo tissue permits evaluation of the impact of the mucus layer on peptide permeability.
- The next steps will be to further evaluate commercial permeability enhancers and formulations in combination with additional clinical peptides and biologics, including PROTACs.