TRANSLATIONAL AND PHSYIOLOGICALLY RELEVENT EX VIVO INTESTINAL SCREENING PLATFORM FOR PREDICTING THE ORAL ABSORPTION OF SMALL MOLECULES AND PEPTIDE FORMULATIONS

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INTRODUCTION

- The oral route is preferred for drug administration
- Predicting human oral absorption remains challenging due to limited translational relevance of animals and conventional *in vitro* models [1]
- Human and porcine intestinal explants better preserve regional structure and key ADME functions
- TNO developed the **InTESTine™**, a physiologically relevant intestinal *ex vivo* tissue model, creating a transwell set-up [2]
- The InTESTine™ model was validated using a training set of small molecules with diverse permeability and transport mechanisms

AIMS

- **To predict human fraction absorbed (F_a) based on P_{app} using *in-vitro in-vivo* correlation (IVIVC)**
- **Demonstrate maintained tissue transporter functionality and Phase I and II metabolic activity**
- **Evaluate the efficacy of clinical permeation enhancers on the intestinal absorption of peptides and proteins**

METHODS

- Porcine or residual human intestinal tissue was stripped of muscle layer and serosa and punched into circular segments) (Figure 1).
- Tissue segments were mounted in the two-compartment InTESTine™ system
- A panel of 24 small molecules with known F_a were applied apically to determine their P_{app} (IVIVC) (Table 1)
- Peptide and protein absorption was evaluated with and without clinically relevant permeation enhancers.

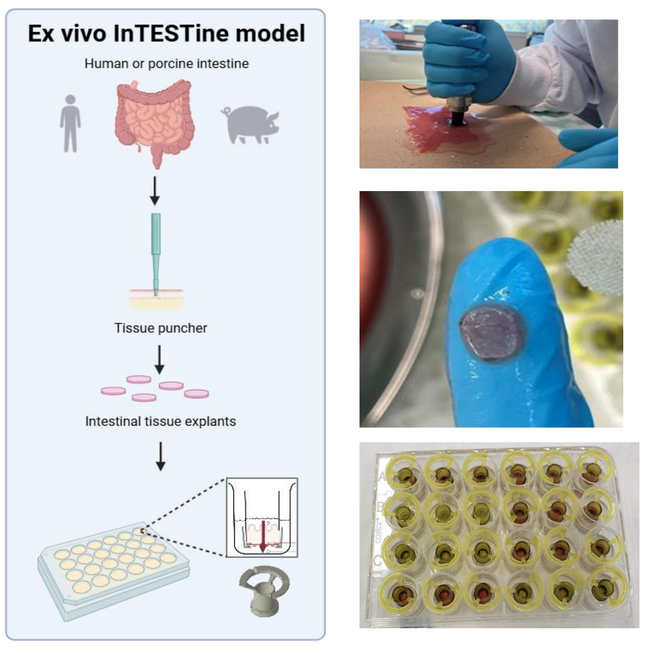

Figure 1. *Schematic representation and photographs of the InTESTine™ model, including tissue processing.*

Table 1. *List of 24 compounds (small molecules) with a known Fa in humans, ranging from high-moderate-low-zero permeability*

Compound	Passive/active transport	Human fraction absorbed ((F_a) (%)	Compound	Passive/active transport	Human fraction absorbed ((F_a) (%)
Caffeine	Passive	100	Metformin	Passive	53
Antipyrine	Passive	99	Atenolol	Passive	51
Warfarin	Active (Efflux)	99	Rosuvastatin	Active (Efflux)	50
Clonidine	Passive	98	Ranitidine	Active	50
Verapamil	Active (Efflux)	98	Acyclovir	Passive	25
Acetaminophen	Passive	85	Methotrexate	Passive	20
Digoxin	Active (Efflux)	81	Mannitol	Passive	19
Creatinine	Passive	80	Sulfasalazine	Active (Efflux)	13
Quinidine	Active (Efflux)	80	FD4	Passive	0
Cimetidine	Active	71	FITC-Inulin	Passive	1
Ciprofloxacin	Active (Efflux)	70	Lucifer Yellow	Passive	NR
Furosemide	Passive	60	PEG 4000	Passive	1

RESULTS

IVIVC of small molecule drugs

- Significant positive rank order relationship between P_{app} values using human and porcine InTESTine™ and *in vivo* F_a
- Predicting F_a (%) in vivo in humans using four parameter logistic (4PL) curve fit
- Permits interpolation of F_a for newly tested compounds using the P_{app} derived from the InTESTine™ model

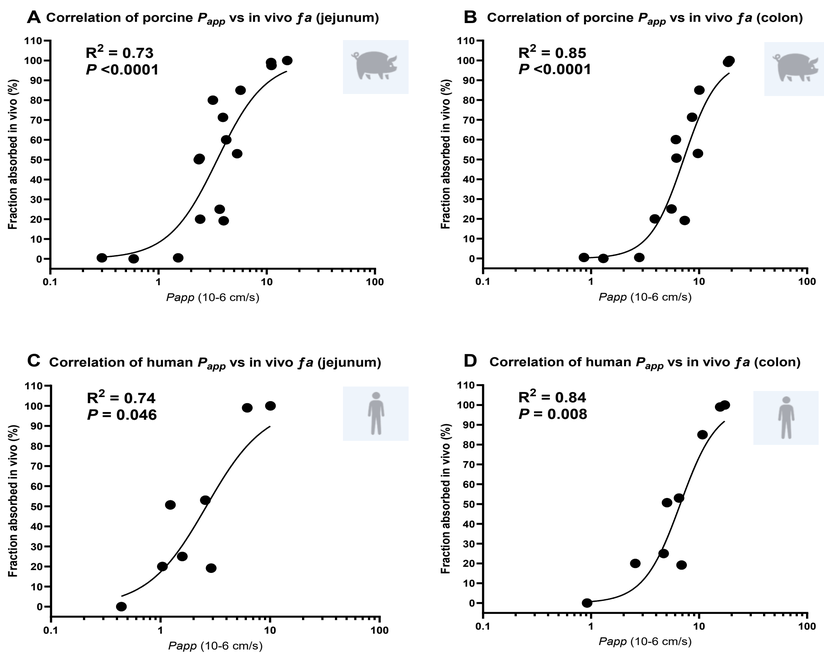

Figure 2: *Correlation plots derived from a 4PL curve fit between the human oral F_a and P_{app} coefficient in porcine (A,B) and human (C,D) jejunum and colon using the InTESTine™ model. Limited tissue availability caused that not all compounds were tested in humans*

Regional absorption

- Drug absorption increases regionally across all permeability classes (duodenum < jejunum < ileum < colon) in both human and pig intestine

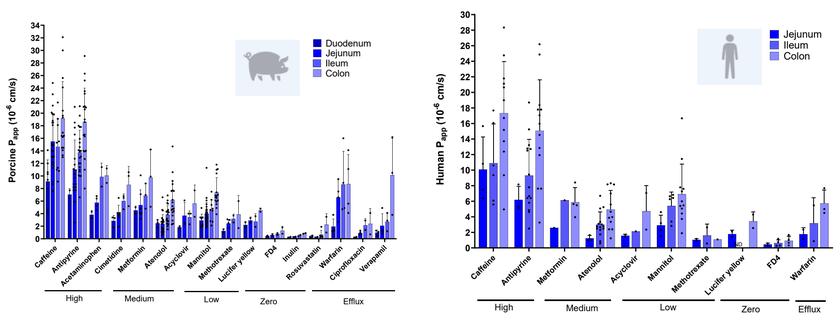

Figure 3: *The P_{app} values derived from porcine and human intestinal tissue. Each data point is an independent biological replicate. Data expressed as mean ± SD*

Intestinal efflux transporters

- Digoxin (P-gp substrate) and rosuvastatin (BCRP substrate) transport tested using porcine ileum tissue
- Quinidine (250 µM) significantly increased digoxin permeability
- Elacridar (10 µM), Ko143 (15 µM), and pantoprazole (300 µM) significantly increased rosuvastatin permeability
- Cyclosporine A (CsA) (100 µM) did not affect rosuvastatin absorption

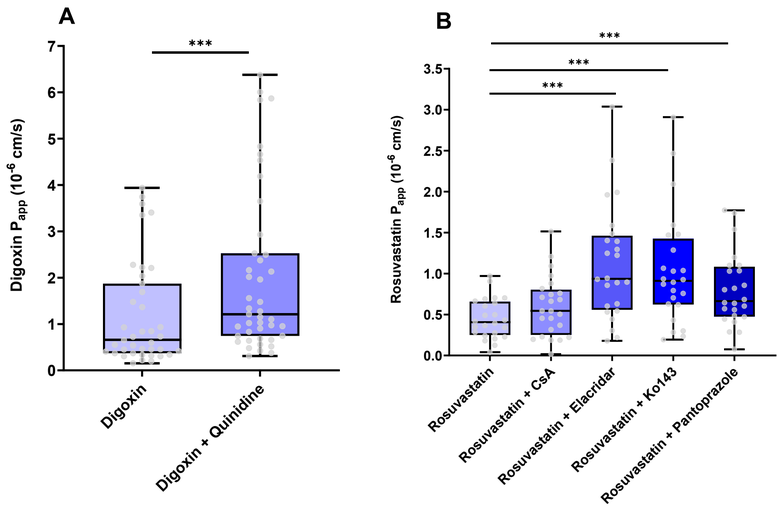

Figure 4: *A) The Papp of P-gp substrate, digoxin in the presence and absence of P-gp inhibitor quinidine. B) The Papp of BCRP substrate, rosuvastatin, in the presence and absence of BCRP inhibitors, CsA, elacridar, Ko143 or pantoprazole. (n = 2-3 donor incubations, all technical replicates shown). Two-way ANOVA with A Šidák multiple comparison correction. ***P<0.001*

RESULTS

Metabolic activity

- Intestinal CYP2A6 Phase I metabolism of coumarin
- Subsequent UGT/SULT-mediated Phase II conjugation of 7-OH-coumarin

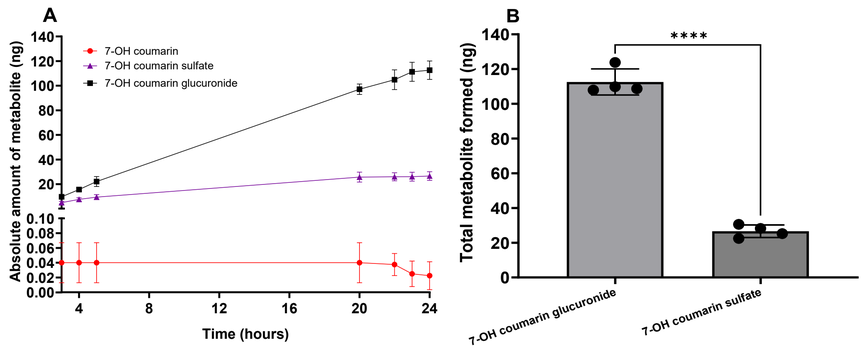

Figure 5: *A) Metabolites (7-OH coumarin, 7-OH coumarin sulfate and 7-OH coumarin glucuronide) measured at each time point over 24 hours B) Total metabolites measured over 24 hours. (n = 1 donor incubation). Student's t-test **** P<0.001*

Permeation enhancer evaluation

- C10 significantly increased the intestinal permeability of oxytocin and insulin, especially at earlier time points
- SNAC enhanced the intestinal permeability of oxytocin only, with delayed onset compared to C10.

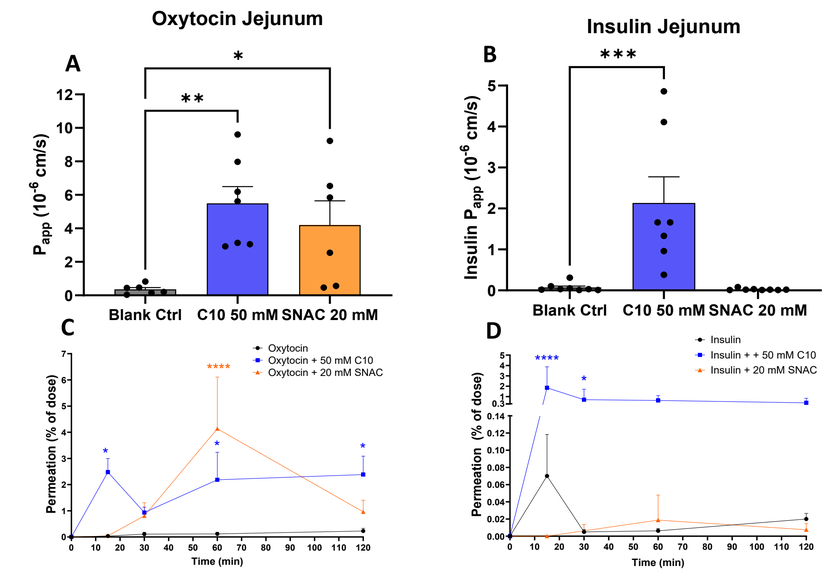

Figure 6: *Porcine jejunum tissue explants in InTESTine™ were exposed to oxytocin (A,C) and insulin (B,D) in the presence and absence of C10 (25 mM) and SNAC (20 mM). (2 donors). Data expressed as mean ± SD. *p<0.05, **P<0.01, ****P<0.0001*

Platform development

- Integration of fluidics in a standardised well-plate format
- Extended tissue viability compared to static InTESTine™

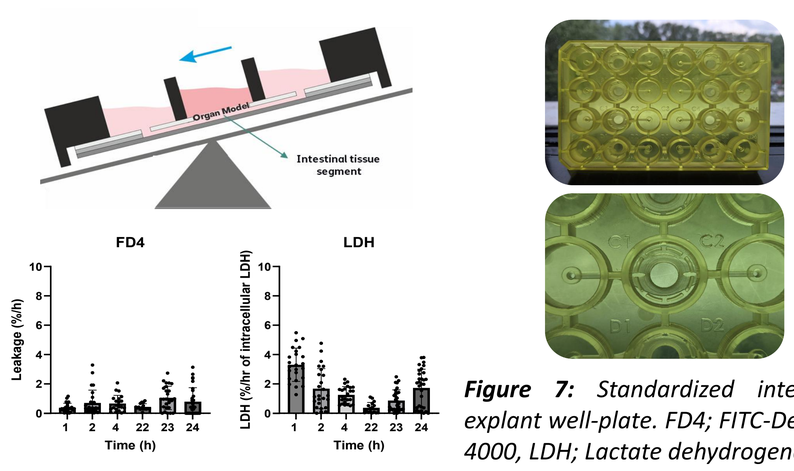

Figure 7: *Standardized intestinal explant well-plate. FD4; FITC-Dextran 4000, LDH; Lactate dehydrogenase*

CONCLUSIONS

- The *ex vivo* InTESTine™ model shows good IVIVC, enabling prediction of human F_a for new compounds
- It supports studies of regional absorption, active transport, and intestinal metabolism.
- InTESTine™ enables evaluation of permeation enhancers by quantifying peptide permeability across the intestinal barrier.
- Next steps include evaluating commercial enhancers and bRo5/PROTAC permeability prediction.

REFERENCES

[1] Keuper-Navis et al.(2023).Pharmacol.Res. DOI:10.1016/j.phrs.2023.106853
[2] Stevens et al.(2019).EJPS. DOI:10.1016/j.ejps.2019.104989