OPTIMIZED NORMOTHERMIC MACHINE PERFUSION OF LIVER AND KIDNEY USED TO PREDICT HUMAN PHARMACOKINETICS

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INTRODUCTION

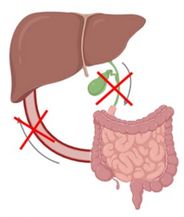
The prediction of hepatic clearance and biliary excretion is of high importance to assess the pharmacokinetics of drugs. Normothermic machine perfusion (NMP) of organs is a promising tool to predict ADME of drugs, especially when combined with physiologically-based pharmacokinetic (PBPK) modeling.

AIM

1) To optimize organ functioning during NMP by improving physiological resemblance of the liver through the addition bile acids
2) To demonstrate prediction of ADME data of model drugs (rosuvastatin, digoxin) based on NMP of liver and kidney combined with ex vivo intestinal absorption studies and PBPK modeling.

METHODS

1) During NMP of the liver the enterohepatic circulation is missing, and standard protocols only apply taurocholate[1,2]

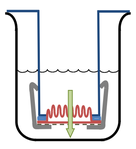


Human (n=4) and porcine (n=5) livers underwent NMP for 360 min using taurocholate.
To study effect of the addition of (un)conjugated bile acids on liver viability and function, unconjugated bile acids (40% gCDCA, 40% gCA, 10% CDCA, 10% CA) were infused for 12 hours of NMP (n=2)

2) Two *ex vivo* models were developed using porcine organs to study ADME processes of rosuvastatin and digoxin

a) Intestinal regional transport

Intestinal transport of rosuvastatin and digoxin was determined using the InTESTine system

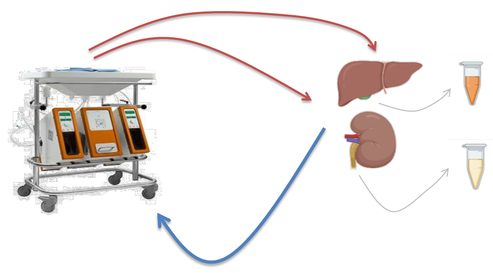


- Porcine jejunum, ileum & colon tissue
- Assessment of apical to basolateral transport

b) Liver & kidney perfusion

Combined liver kidney perfusion was performed to study biliary end renal clearance

- Cannulation hepatic artery & portal vein (liver) renal artery (kidney)
- Slow bolus dosing via portal vein
- Perfusate, bile and urine samples taken over time



c) PBPK modelling

A generic PBPK model was developed and combined with the input of drug and system specific properties

Ex vivo models input:

- InTESTine: regional Papp data
- Liver & kidney perfusion derived data

RESULTS

1) Solely taurocholate infusion during NMP

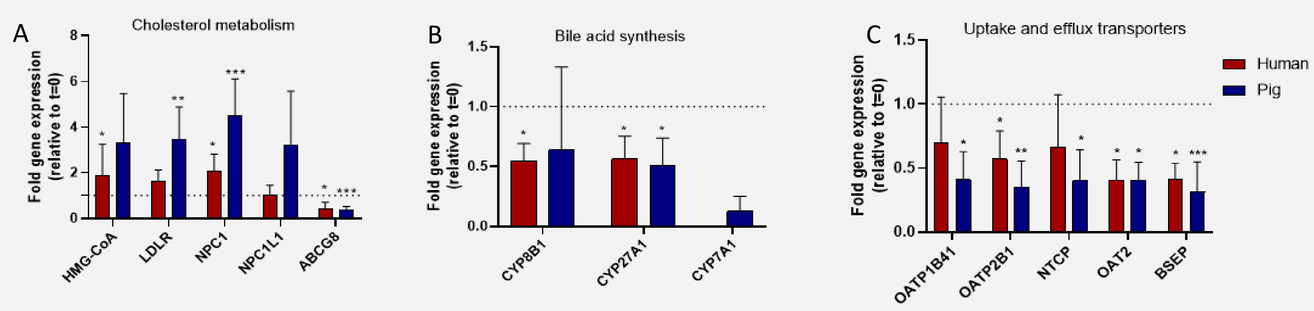


Figure 1. Changes in gene expression after hours of NMP in human (n=4) and porcine (n=5) livers. *Genes related to (A) Cholesterol metabolism (B) Bile acid synthesis and (C) Uptake and efflux transporters. Fold changes was calculated as expression relative to t=0h. Significance was measured by paired t-test, *p<0.05, **p<0.01, ***p<0.001.*

2) Infusion of (un)conjugated bile acids during NMP

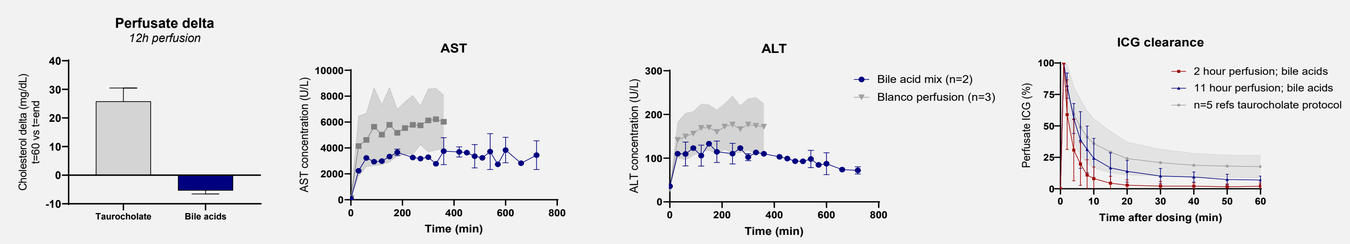
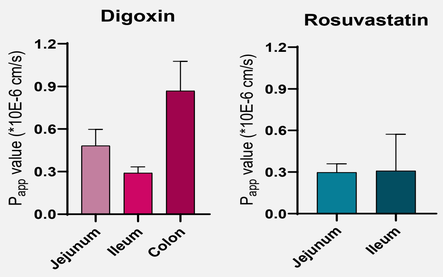


Figure 2. Effect of infusion of a (un)conjugated bile acid pool during 12 hours of NMP of porcine liver (n=3). *(A) Perfusate cholesterol concentration in taurocholate (normal conditions) vs. Infusion of bile acids), (B) AST and (C) ALT perfusate concentrations during perfusion (D) ICG clearance from the perfusate at 2 and 11 hours of perfusion compared to reference perfusions (taurocholate protocol).*

3) Intestinal transport

Basolateral sample



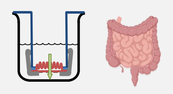
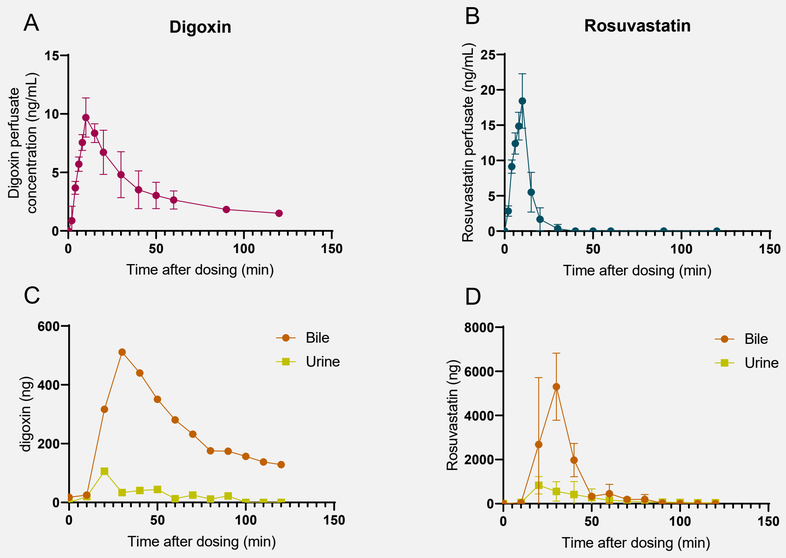


Figure 3. Intestinal absorption of rosuvastatin and digoxin. *Papp values in jejunum, ileum and colon of (A) Digoxin and (B) rosuvastatin. Data represents mean ±SD (n=3).*

4) Hepatic, biliary and renal clearance

Perfusate

Bile Urine



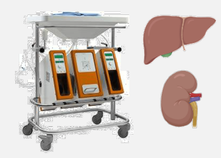


Figure 4. Hepatic and renal clearance of digoxin and rosuvastatin *(A) Perfusate clearance of digoxin and (B) rosuvastatin. Biliary and urinary fractions were measured for (C) digoxin and (D) rosuvastatin. Data represents mean ±SD (n=2).*

5) PBPK modelling

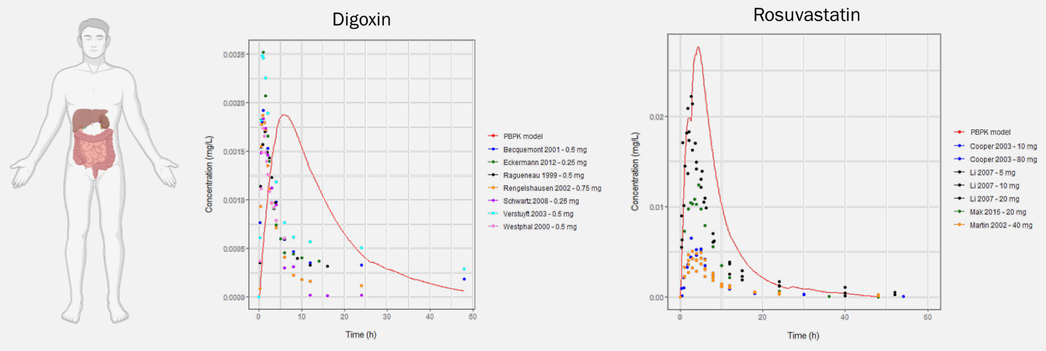


Figure 5. *Simulated plasma profile of (A) digoxin and (B) rosuvastatin compared to clinical data.*

Table 1. *The simulated vs. clinical pharmacokinetic parameters for single PO dose of 0.5 mg digoxin or 10 mg rosuvastatin*

	Parameter	Simulated	Clinical data
Digoxin	C_{max} *(ng/mL)*	1.90	2.50 ± 0.70
	T_{max} *(h)*	5.80	1.50 (0.8-2.3)
	*AUC (h*ng/mL)*	32.1	28.3 ± 6.3
Rosuvastatin	C_{max} *(ng/mL)*	27.7	25.9 ± 18.77
	T_{max} *(h)*	4.40	3.91 ± 3.73
	*AUC (h*ng/mL)*	255.6	210.2 ± 178.70

- Ex vivo data was incorporated into a generic PBPK model and simulations resulted in relatively accurate predictions of the plasma concentration (Area Under the Curve (AUC)), plasma peak concentration (C_{max}), hepatic clearance and bioavailability when compared to human clinical studies

REFERENCES

1. Eshmuminov, D et al., (2018). Perfusion settings and additives in liver normothermic machine perfusion with red blood cells as oxygen carrier. A systematic review of human and porcine perfusion protocols. *Transplant International*, *31*(9), 956-969.
2. Lascaris, B et al., (2022). Long-term normothermic machine preservation of human livers: what is needed to succeed?. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *322*(2), G183-G200.

CONCLUSIONS

- 1) Portal infusion of (un)conjugated bile acids resulted in improved liver functions and cholesterol metabolism
- 2) The combination of ex vivo gut, liver and kidney models with a generic PBPK model is a unique and powerful combination to predict ADME profile of (new) drugs, with the possibility to calculate the fraction that undergoes enterohepatic circulation.

Future research is aimed at studying drug-drug interactions and the effects of disease processes.