PHARMACOKINETIC APPLICATION OF NORMOTHERMIC PERFUSED EX VIVO PORCINE LIVERS

Lianne Stevens[1,2]
Jeroen Dubbeld[2]
Jason Doppenberg[2]
Steven Erpelinck[1]
Catherijne Knibbe[3]
Ian Alwayn[2]
Evita van de Steeg[1]

[1]TNO, Microbiology & Systems Biology, The Netherlands
[2] Leiden University Medical Centre, The Netherlands
[3] Leiden Academic Centre Drug Research, The Netherlands

E: lianne.stevens@tno.nl

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INTRODUCTION

Current models to predict biliary excretion often fail due to species differences (rodent/dog) or due to differences in transporter expression in in vitro assays (e.g. sandwich cultured hepatocytes). Especially when drugs are subjective to enterohepatic circulation (EHC), this results difficulties to predict plasma profiles after oral and iv administration. Moreover, in case a compound is subjective to EHC, it is more prone to cause any drug-drug interaction and/or drug induced liver injury.

AIM

We aimed to set-up a pre-clinical model to study hepatic clearance, biliary excretion and the effect of drug-drug interaction on these processes by applying whole porcine liver on a pressure-controlled perfusion machine (Liver Assist).

METHODS

Porcine livers were made available from the slaughterhouse. After termination, the portal vein and hepatic artery were cannulated and flushed with saline and HTK solution. Bile duct was cannulated and liver was connected to the LiverAssist (figure 1A and B). Livers were perfused under oxygenated (95% O2, 5% CO2) conditions at 37°C. Perfusate composition is shown in Table 1. A bolus injection of the drug of interest was applied at the portal vein and samples were taken in time of the perfusate and the bile. Every hour bloodgas analysis was performed to measure the functional and metabolic status of the liver.

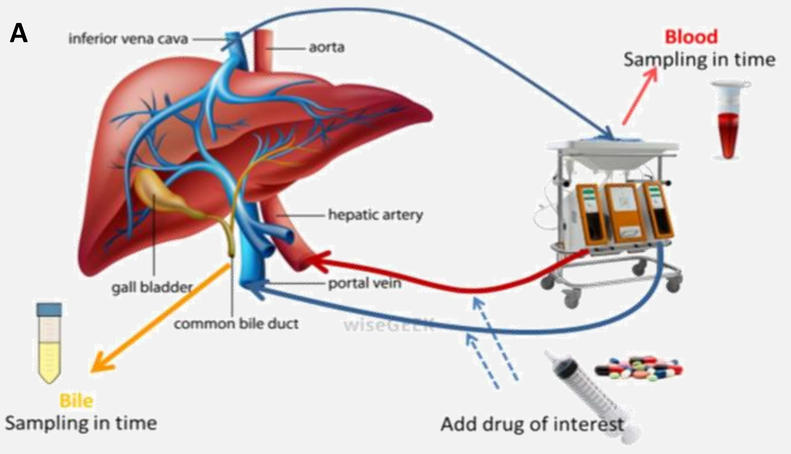
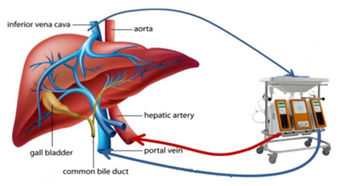


***Figure** 1. (A) Schematic representation of the liver connected to the LiverAssist and (B) the LiverAssist set up with the porcine liver in the laboratory*

Table 1. Perfusate composition of the perfused liver



Perfusate	
Red blood cells	1L
Plasma	1L
Sodium bicarbonate 8,4%	30 mL
Calcium gluconate 10%	10 mL

Continuous infusion	
Insulin	100U/hr
Epoprostenol	8 µg/h
Taurocholate	2%w/v / h

RESULTS

Functional and metabolic parameters measured during normothermic perfusion

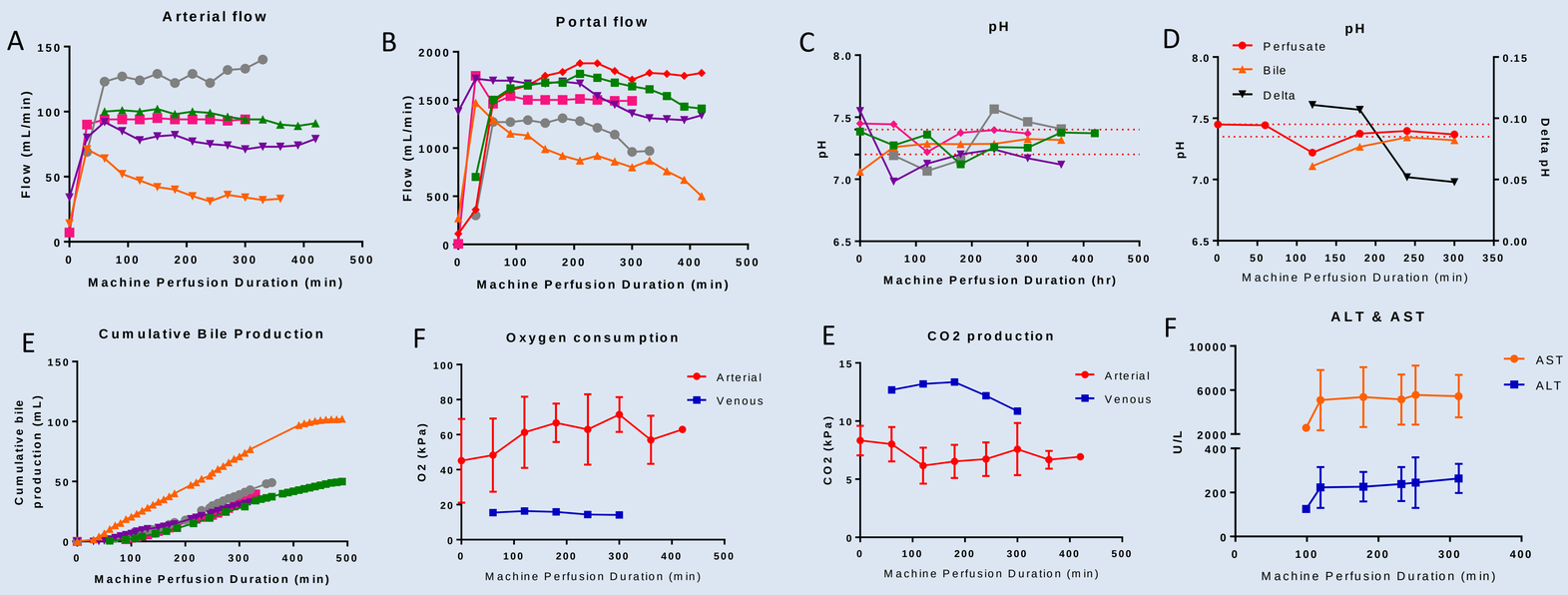


Figure 2. Parameters shown during ex vivo porcine liver perfusion. (A) Livers are perfused via the Hepatic Artery (B) and the Portal Vein (C) during perfusion, perfusate pH is monitored and shows stable pH values. (D) livers showed to produce bile (E) pH of the perfusate and bile is measured to study the viability of the perfused liver (F) The livers showed to consume oxygen while perfused under normothermic conditions

Transporter interactions

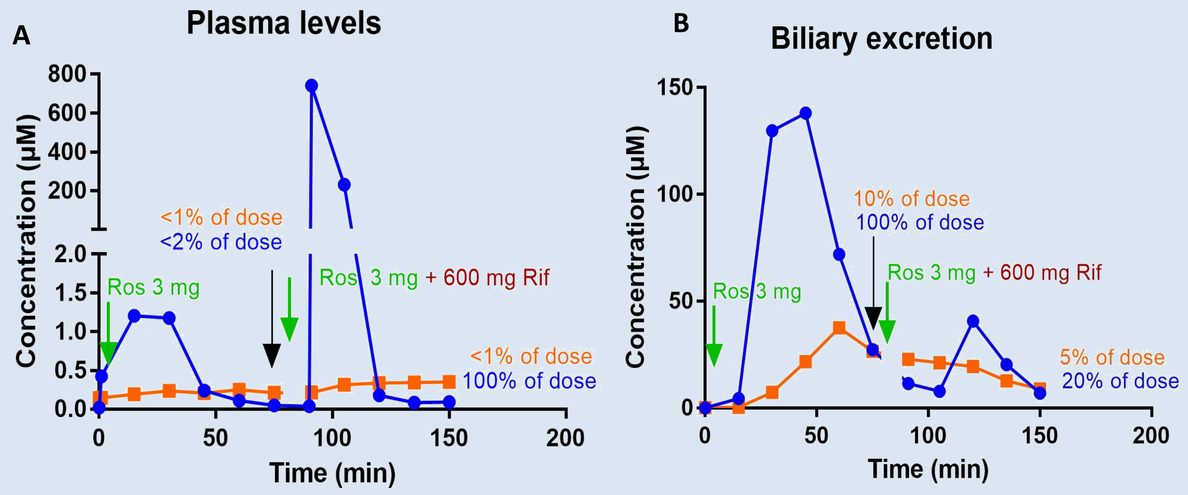


Figure 3. Plasma kinetics (**A**) and biliary excretion (**B**) of rosuvastatin and N-desmethyl rosuvastatin for 180 min after bolus injection of 3 mg rosuvastatin to ex vivo cannulated and perfused porcine livers in absence and presence of hepatic uptake and biliary excretion transporter inhibitor rifampicin (600 mg/h, continuous infusion).

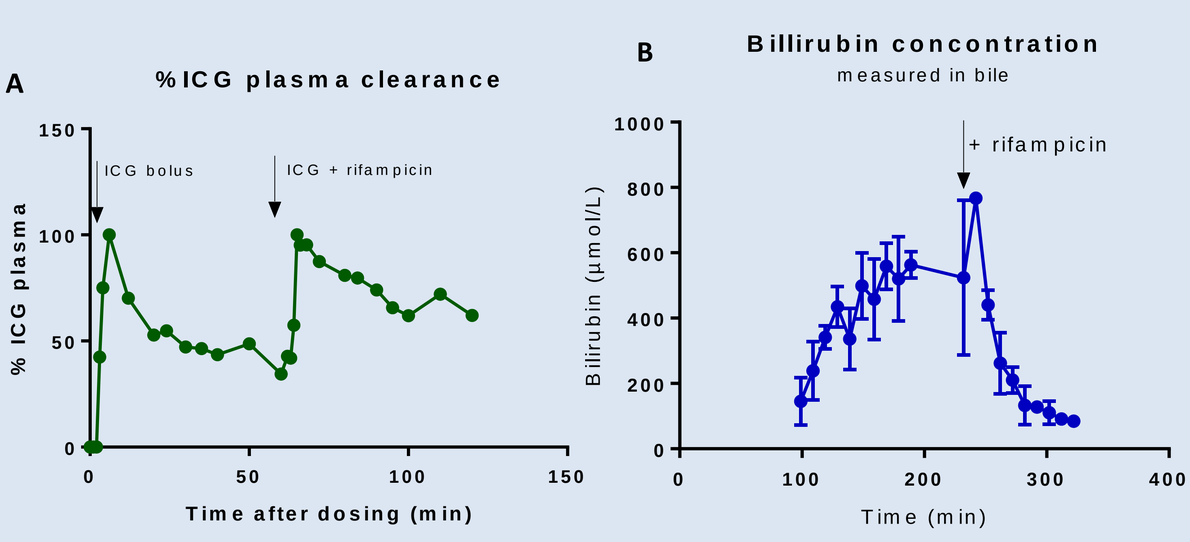


Figure 4. (A) Plasma clearance of indocyanine green (ICG) after a bolus injection and after 60 min a bolus injection + 25mg rifampicin showing delayed plasma clearance. (**B**) bilirubin concentration in the bile after a bolus + continuous infusion of 600 mg Rifampicin.

CONCLUSIONS

- The porcine liver was functional and metabolically active for at least 6h while being perfused under oxygenated and normothermic conditions.
- We have demonstrated the feasibility of NMP of porcine livers as a valuable tool to study hepatic clearance and biliary excretion of rosuvastatin, which was used as a model drug.

FUTURE PERSPECTIVES

Currently we are investigating the effect of longterm liver perfusion on transporter and enzyme expression and activity.