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EX VIVO WHOLE LIVER PERFUSION MODEL FOR PREDICTION OF DRUG-DRUG INTERACTIONS AND BILIARY EXCRETION OF ROSUVASTATIN

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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.

Goal

In order to study the feasibility to set-up a preclinical model to investigate hepatic clearance, biliary excretion and the effect of drug-drug interaction on these processes, we have applied whole porcine liver on a pressure-controlled perfusion machine (Liver Assist).

Approach

Prior to isolation of the liver from the anaesthetized pigs, the blood was heparinized, portal vein and hepatic artery were cannulated and directly after cutting the hepatic inferior vena cava the liver was flushed with warm Ringers buffer. Bile duct was cannulated directly after positioning of the liver on the Liver Assist device, on which it underwent normothermic perfusion at 37°C. Rosuvastatin (3 mg bolus injection) was used a model compound to study hepatic clearance and biliary excretion in the absence and presence of rifampicin (600 mg/h, continuous infusion). Blood samples were taken 15, 30, 45 and 60 minutes after dosing and bile was collected in 15 min fractions.

Results

First steps in development of this ex vivo perfused porcine liver model:

- Demonstrator study showing feasibility of the application of ex vivo perfused porcine liver as good model to study hepatic clearance and biliary excretion of drugs
- In the presence of rifampicin, biliary excretion of rosuvastatin was decreased to 20% of the dose (whereas 100% when rosuvastatin was dosed alone), and plasma AUC was 200-fold increased

Ex vivo model to study hepatic clearance and biliary excretion of drugs

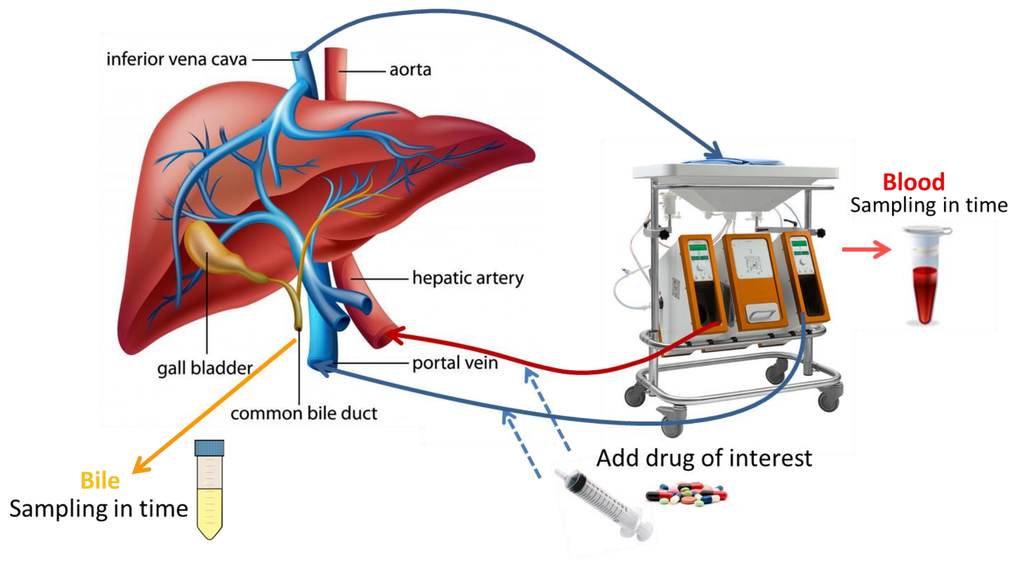

Fig 1. Schematic representation of the ex vivo perfused liver set-up on a pressure-controlled perfusion machine (LiverAssist) as applied in the current study.

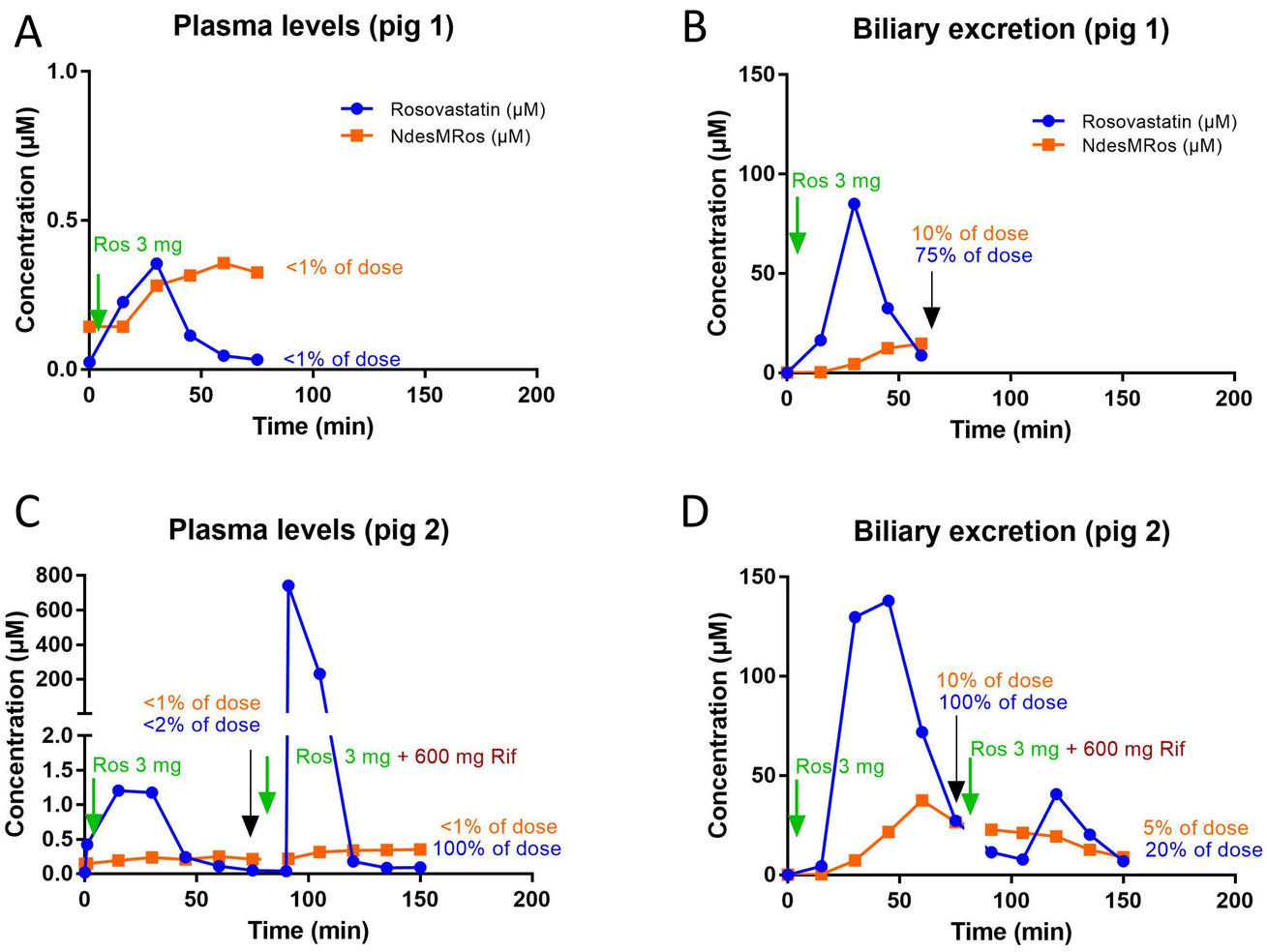

Fig 2. Plasma kinetics (**A,C**) and biliary excretion (**B,D**) of rosuvastatin and N-desmethyl rosuvastatin for 180 min after bolus injection of 3 mg rosuvastatin to ex vivo cannulated and perfused porcine livers in absence and presence of hepatic uptake and biliary excretion transporter inhibitor rifampicin (600 mg/h, continuous infusion).

Conclusions / next steps:

- We have successfully developed an ex vivo liver perfusion model to study hepatic clearance and biliary excretion of drugs and the effect of drug-drug interaction on these processes.
- Next studies will be focused on studying hepatic metabolism, the effect of long-term liver perfusion on hepatic transporter expression and the possibility to apply diseased human livers which become redundant after liver transplantation.
- For these projects TNO is looking for partners to further co-develop these models:

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Milestones include:

- Ethical approval for the use of **human (NASH/cirrhotic) livers** that become redundant upon liver transplantation (METC approval, informed consent)
- Set-up of ex vivo perfused human NASH liver model (N=4 to study feasibility, reproducibility, variation)
- Demonstrator study (n=2): PK & biliary excretion with cocktail of clinically relevant compound (e.g. Digoxin, Furosemide, Metformin, and Rosuvastatin)
- **Determine liver PK, metabolism and biliary excretion (and target binding) of 1 compound or cocktail of compounds that is brought in by each of the pharmaceutical partners (n=2). Compare with clinically available data (validation).**
- Investigation of effect of compound on relevant biomarkers (online and/or after-study)
- Other parameters to be measured by clinic chemistry and/or analytical chemistry methods

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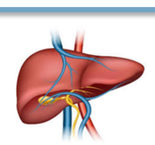

Milestones include:

- Set-up of ex vivo perfused **healthy porcine liver** model to predict effect of DDI on biliary excretion and plasma exposure of API & metabolites:
  - Demonstrator study: PK & biliary excretion of a clinically relevant DDI (n=4)
- Determine effect of perfusion on expression levels of relevant transporter proteins and metabolizing enzymes
- Explore possibilities to increase functionality of the liver > 24 hours
- Develop PBPK model to predict plasma kinetics upon iv dose (distribution volume, urinary excretion)
- **Determine liver PK, metabolism and biliary excretion of 1 compound (or cocktail of compounds) that is brought in by each of the pharmaceutical partners (n=2). Compare with clinically available data (validation).**