The impact of P450 oxidoreductase knock out on systemic exposure to rosuvastatin, atorvastatin and atorvastatin metabolites

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Introduction

- **Statins** are amongst the most commonly prescribed medications worldwide.
- Although statins are generally well tolerated, **myotoxicity** likely attributable to statin therapy occurs in ~5% of patients[1].
- Atorvastatin (ATV) is hydroxylated by **CYP3A4**
    - Concomitant CYP3A4-inhibiting drug therapy is an established ATV myotoxicity risk factor[2].
- Rosuvastatin (RVT) is metabolised only to a minor extent, and mainly by CYP2C9 (~10%)[3].
- **P450 oxidoreductase (POR)** is the major electron donor for all microsomal CYP enzymes[4]; importantly, its effects on statin pharmacokinetics are unknown.

Aim

- This work aimed to determine whether POR deficiency (knock out) alters statin exposure using the **selective hepatic POR null murine model (HRN).**

Results

1.) The *in vitro* liver microsome incubations demonstrated that POR deficiency is associated with:
- **decreased ATV hydroxylation (Figure 1),**
- **no effect on RVT hydroxylation (Figure 2).**

2.) A corresponding significant **increase** in **ATV** *in vivo* blood maximum concentration (Figure 3) and exposure (Figures 3 &4) in HRN mice was observed.

3.) Unexpectedly, a significant **increase** in the *in vivo* blood maximum concentrations (Figure 3) and exposures (Figures 3 & 4) of **all ATV metabolites** and **RVT** in HRN mice was also observed.

Figure 1 Hepatic microsomal POR-dependent ATV hydroxylation

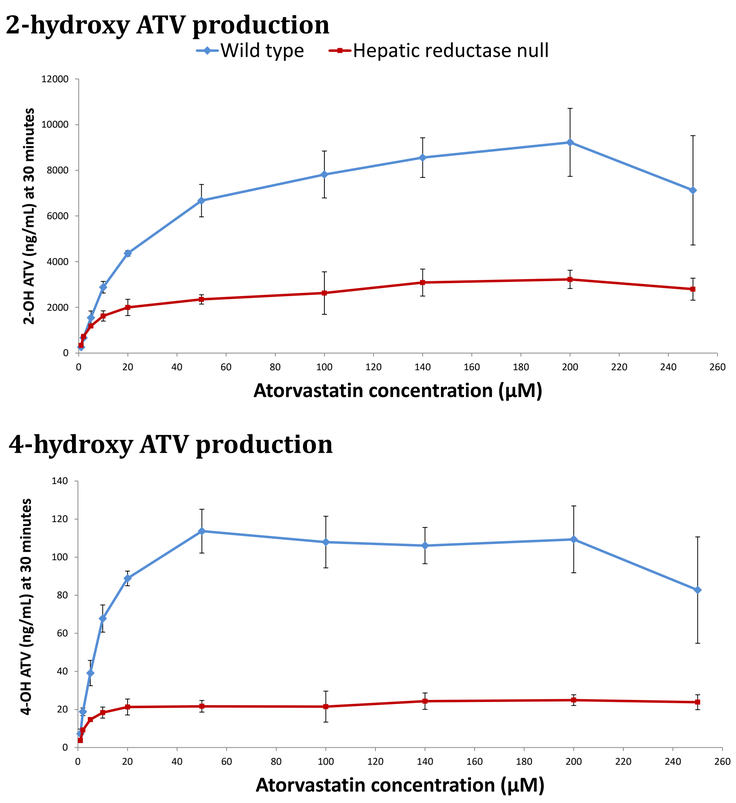
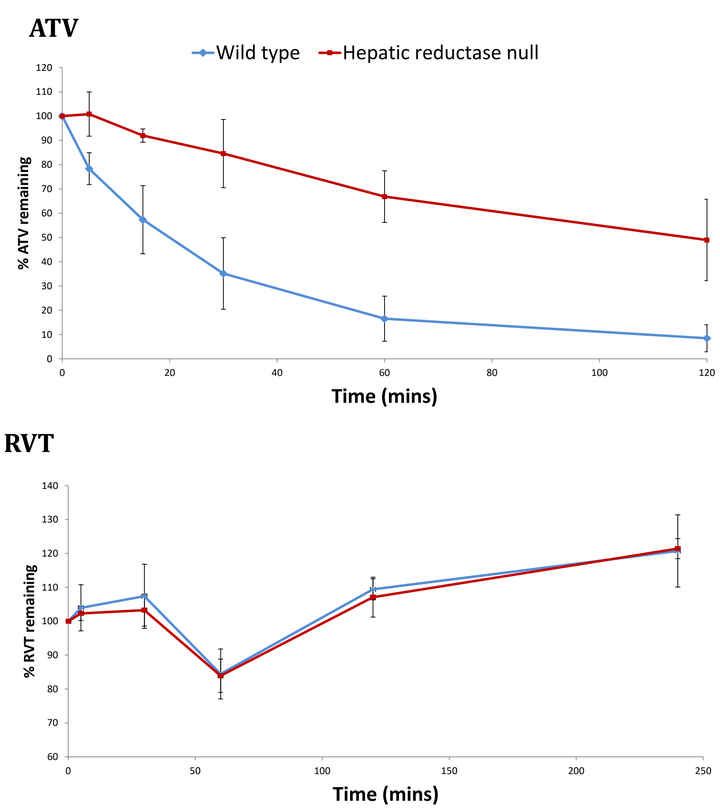


Figure 2 Proportions of 20µM ATV and RVT remaining in microsomal incubations



Discussion

1.) These results demonstrate that POR deficiency is associated with:
- reduced *in vitro* ATV hydroxylation, likely reflecting decreased Cyp3a activity
- no difference in *in vitro* RVT levels, reflecting the minimal contribution of Cyp to RVT disposition.

2.) The *in vivo* increase of ATV lactone may represent a compensatory increase in non-POR dependent UDP-glucuronyltransferase activity in HRN livers.

3.) The unexpected increase in hydroxy-ATV metabolites and RVT exposures in HRN mice *in vivo* suggests extra-hepatic CYP3A-mediated hydroxylation, an effect of transporters or potentially reduced hepatic uptake due to the fatty liver that develops in HRN mice.

4.) Further research to characterise transporter protein expression in HRN mice is ongoing.

Methods

1.) Hepatic microsomes from three wild-type (WT) and three HRN male mice were incubated (200µL final volume) for:
a) 30 minutes with ATV (1-250µM) and;
b) up to 120 minutes (ATV) or 240 minutes (RVT) at 20µM.

2.) Separately, three WT and three HRN male mice each received 30mg/Kg ATV and 30mg/Kg RVT together via intraperitoneal injection (10mL/Kg).
- Serial blood samples were collected up to 24 hours onto dried blood spots.
- The study was carried out in accordance with the Animal Scientific Procedures Act of 1986 and after a local ethics review.

All bioanalysis was by liquid chromatography-mass spectrometry (Sciex 6500) using a method validated according to the FDA guidelines (2001). Pharmacokinetic analysis used the Real Statistics Resource Pack Excel add-in; statistical analysis was by Student's t-test ($p<0.05$ designated as significant).

Figure 3 *In vivo* pharmacokinetic profiles

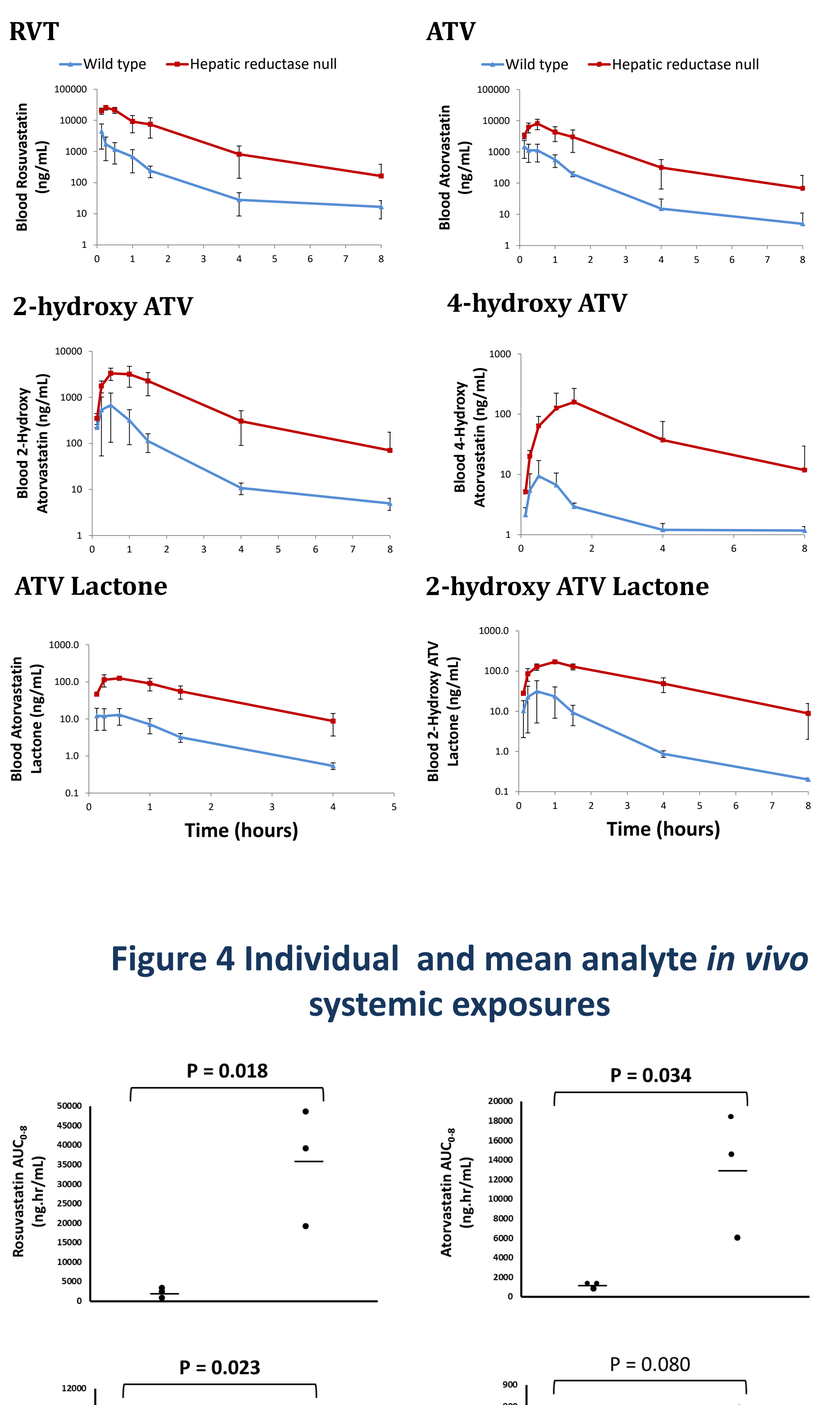
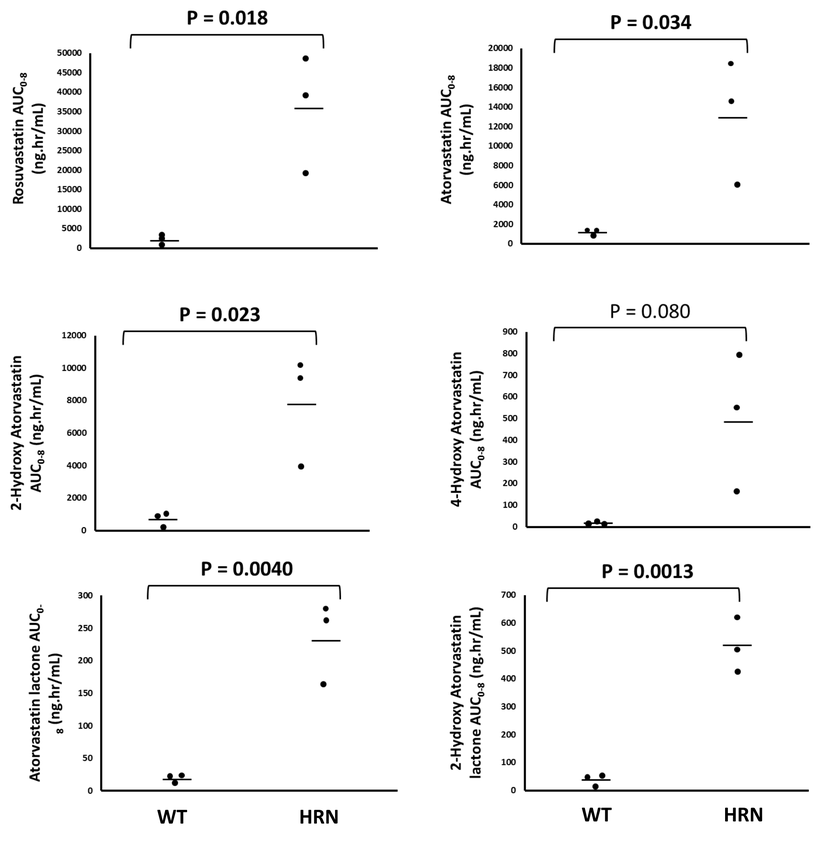


Figure 4 Individual and mean analyte *in vivo* systemic exposures



References
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