Title: Assessing drug-mediated inhibition of liver transporter function with MRI: A first-in-human study

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Abbreviated title page

Title of the manuscript: Assessing drug-mediated inhibition of liver transporter function with MRI: A first-in-human study

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Summary statement:

The biliary excretion rate and hepatocellular uptake rate of gadoxetate are promising biomarkers for research into drug-mediated inhibition of hepatocellular transporter function.

List of abbreviations

DILI: Drug-induced liver injury

DDI: Drug-drug interaction

DGE-MRI: Dynamic gadoxetate-enhanced MRI

CI: Confidence interval

LFT: Liver Function Test

MOLLI: modified Look-Locker sequence

k(he): Hepatocellular uptake rate

k(bh): Biliary excretion rate

Introduction

Drug induced liver injury (DILI) and drug-drug interactions (DDIs) often arise from drug effects on liver transporter function, and may result in liver failure or impact on treatment efficacy and toxicity [1,2]. Understanding these effects for novel drugs is critical to minimize the risk of drug toxicity, but *in-vitro* and *in-silico* results are difficult to verify clinically, especially when the drug inhibits biliary excretion [3,4].

Studies in healthy rats have shown that dynamic gadoxetate-enhanced MRI (DGE-MRI) can measure drug-mediated inhibition of liver transporter function reproducibly, and critically can distinguish inhibition of hepatocellular uptake from biliary excretion [5]. This proof-of-concept study aimed to test whether these findings translate to humans by measuring the effect of rifampicin, a known potent inhibitor drug.

Materials and Methods

This prospective study was approved by the East Midlands Nottingham 1 research ethics committee (reference 21/EM/0118), and recruited 10 healthy volunteers without regular prescribed medication or history of liver- or kidney disease.

Each participant underwent two study visits, 2-4 weeks apart (Figure 1). On visit 2, 600 mg rifampicin was administered orally, one hour before MRI. DGE-MRI was performed over two scans each visit to capture the slow excretion after inhibition. Blood samples were taken for liver function tests (LFTs).

MRI was performed on a 3T Siemens Prisma and included T1-mapping and coronal 3D free-breathing DGE-MRI for 50 min at 2.3s per volume. Gadoxetate was injected at 1/8th of a clinical dose for the first 2 participants, and 1/4th for the others. Whole liver and aorta were segmented manually after retrospective motion correction, and signals were analysed using the function AortaLiver2scan in the python package *dcmri* [6].

A two-sided paired t-test was used to test for a drug effect on the uptake rate k(he) from extracellular space (e) to hepatocytes (h), and on the excretion rate k(bh) from hepatocytes to bile (b). Secondary explorative endpoints were correlation of effect sizes between k(he), k(bh), and LFTs. $p<0.01$ was considered significant for all tests.

Results

Volunteers (3/10 female) had (mean +/- stdev) age 32 +/- 8.3, BMI 24 +/- 3.5 and normal LFTs (ALP 68 +/- 26 U/L, ALT 21 +/- 8.2 U/L, Bilirubin 13 +/- 4.2 umol/L). 8 participants completed both visits and LFTs did not change significantly between visits. No incidental findings were noted.

Rifampicin reduced k(he) by 93% (95%CI 91-95%, $p<0.001$), k(bh) by 50% (95%CI 8-92%, $p=0.004$), and bilirubin by 82% (95%CI 30-134, $p=0.001$). The other LFT's showed no effect. k(he) and k(bh) reduced in each individual, except k(bh) in one participant with low baseline k(bh) (Figure 2). Changes in k(he) and k(bh) were not significantly correlated, and were uncorrelated with changes in LFT's.

Conclusion

The results confirm expectations that inhibition by rifampicin in humans is similar to healthy rats, which showed a 90% reduction in k(he) and 43% in k(bh) [5]. This supports the use of the assay as a tool for clinical translation in drug toxicity research, and provides a rationale for more in-depth characterization with different drugs, doses, and levels of liver function.

Exploratory analyses suggest the assay has an added value over LFTs and can differentiate inhibition of uptake from excretion. The between-subject variability also points to a gap in knowledge about the heterogeneity of liver transporter function in humans, and suggests the need of a personalised approach to DDI and DILI prevention.

A key limitation of the assay is that it only detects inhibition of hepatic transporters for which gadoxetate serves as a substrate, such as OATP1B1 and MRP2. This study was powered for the primary endpoints but not for the exploratory analyses, which are hypothesis-generating. While the study supports a utility in drug research and development, potential use in clinical practice remains an open question.

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Figures

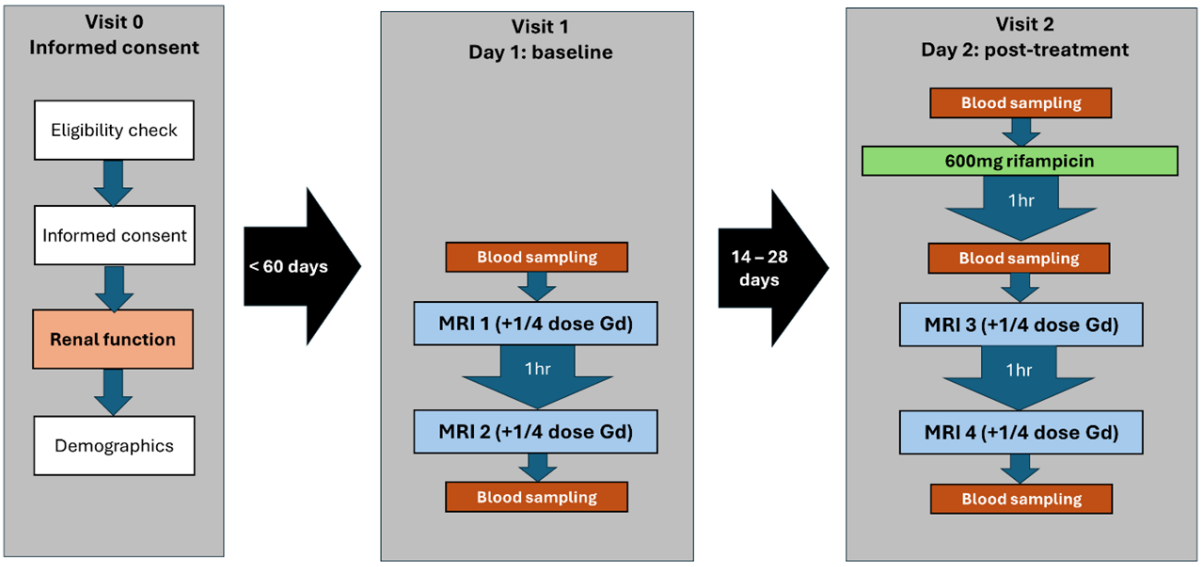

Figure 1: Flow diagram of the final protocol after dose escalation, showing the study design and assessments performed at each visit. Visit 0 was a screening visit to assess eligibility for the study, take informed consent and collect key data. Visit 1 was the baseline visit, no more than 60 days after consent. It involved two MRI scans at least 2 hours apart, and blood tests before and after the scans. The post-treatment visit 2 took place between 2 and 4 weeks after the baseline visit, and involved blood sampling before oral rifampicin administration, then after 1 hour a repeat of the same protocol performed at visit 1.

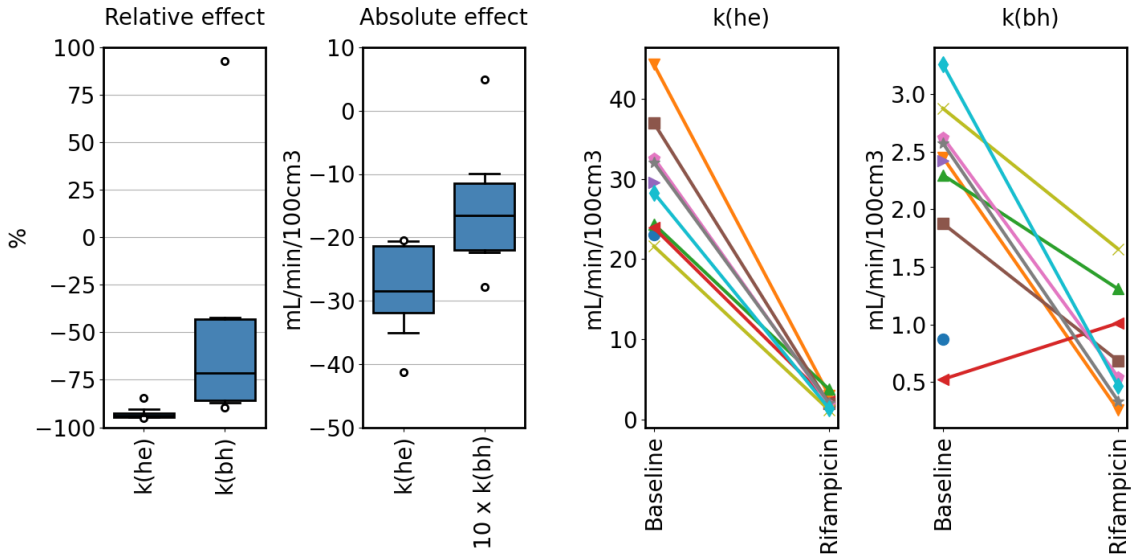

Figure 2. Rifampicin effect on the primary endpoints k(he) (hepatocellular uptake rate) and k(bh) (biliary excretion rate) across the population. Box plots on the left show the relative and absolute effect size across the population. Absolute effect sizes for k(bh) have been scaled with a factor 10 to improve visualisation. Line plots on the right show individual values at baseline and after single dose of rifampicin, with each line representing an individual participant. Values for participants that attended the baseline visit only are shown with a single plot symbol.