Modelling Gadoxetate Liver Uptake and Efflux Using DCE-MRI Enables Preclinical Quantification of Transporter-Mediated Drug-Drug Interactions

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Investigative Radiology

Modelling Gadoxetate Liver Uptake and Efflux Using DCE-MRI Enables Preclinical Quantification of Transporter-Mediated Drug-Drug Interactions --Manuscript Draft--

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Abstract:	<p>Objectives: To model the in vivo transporter-mediated uptake and efflux of the hepatobiliary contrast agent, gadoxetate, in the liver. The efficacy of the proposed technique was assessed for its ability to provide quantitative insights into drug-drug interactions (DDIs), using rifampicin as inhibitor.</p> <p>Materials and Methods: Three groups of C57 mice were scanned twice with a gadoxetate-enhanced dynamic Magnetic Resonance Imaging (DCE-MRI) protocol, using a 3-dimensional spoiled gradient-echo sequence for approximately 72 minutes. Before the second MRI session, two of the groups received a rifampicin dose of 20 (n=7) or 40 (n=7) mg/kg, respectively. Data from regions of interest in the liver were analysed using two simplifications of a two-compartment uptake and efflux model to provide estimates for the gadoxetate uptake rate (k_i) into the hepatocytes and its efflux rate (k_{ef}) into the bile. Both models were assessed for goodness-of-fit in the group without rifampicin (n=9) and the appropriate model was selected for assessing the ability to monitor DDIs in vivo.</p> <p>Results: Seven out of nine mice from the group without rifampicin were assessed for model implementation and reproducibility. A simple three parameter model (k_i, k_{ef}, and extracellular space, $vecs$) adequately described the observed liver concentration time series with mean $k_i = 0.47 \pm 0.11 \text{ min}^{-1}$ and mean $k_{ef} = 0.039 \pm 0.016 \text{ min}^{-1}$. Visually the area under the liver concentration time profile was reduced for the groups receiving rifampicin. Furthermore, tracer kinetic modelling demonstrated a significant dose-dependent decrease in the uptake (5.9 and 17.3 fold decrease for 20mg/kg and 40mg/kg, respectively) and efflux rates (2.2 and 7.9 fold decrease) compared with the first scan for each group.</p> <p>Conclusions: This study presents the first in vivo implementation of a dual-input two-compartment uptake and efflux model to monitor DDIs at the transporter-protein level, using the clinically-relevant OATP inhibitor, rifampicin. The technique has the potential</p>

	to be a novel alternative to other methods, allowing real-time changes in transporter DDIs to be measured directly in vivo.
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Response to reviewers

Thank you for your positive response to our manuscript. We would like to thank the reviewers for their useful and insightful comments. Please find our revisions attached (with altered text in red) and commented upon below.

Reviewers' comments:

Reviewer #1: This study investigates pharmacokinetic models to estimate Gadoxetate transport in mice liver and quantify how rifampicin alters Gadoxetate transport across OATP and MRP2

Title

The reviewer suggests a slightly modified title: "Modelling Gadoxetate Liver Uptake and Efflux Using DCE-MRI Enables Preclinical Quantification of Transporter-mediated Drug-Drug Interactions"

We have modified the title accordingly.

Abstract

1. "Qualitatively, the area under the liver concentration time profile was reduced for the groups receiving rifampicin." The reviewer does not understand while liver concentrations over time are qualitative and not quantitative data.

We have changed "qualitatively" to "visually" (page 1).

2. "Conclusions: This study presents the first in vivo implementation of a dual-input two compartment uptake and efflux model to monitor DDIs at the transporter-protein level, using the clinically-relevant OATP inhibitor, rifampicin." The model is "based" on a dual-input two compartment uptake and efflux model.

We have removed the term "dual-input" for clarity (page 2).

Introduction

1. "It is known that interactions of drugs with membrane transporters can result in altered drug levels in tissues and/or blood, which in turn may have implications for both safety and efficacy". Rather that levels, concentrations might be more appropriate.

We have changed "levels" to "concentrations", as suggested (page 3).

2. "Transporter-mediated DDIs at the level of the liver therefore have the potential to significantly alter the pharmacokinetic profile of administered drugs, affecting drug safety and efficacy". Authors should replace level by concentrations and state that drug efficacy is link to adequate concentrations at the site of action, while safety might be altered when concentrations are high (potential toxicity).

Level here doesn't refer to concentration. We have reworded this sentence and included a definition of drug safety and efficacy, as suggested.

"Transporter-mediated DDIs in the liver therefore have the potential to significantly alter the pharmacokinetic profile of administered drugs and, if drug levels are altered, may impact drug efficacy (if concentrations at the site of action are not adequate for effect) and/or drug safety (if concentrations are too high, leading to potential toxicity)." (page 4).

3. "However, predicting the severity of clinically-relevant DDIs from in vitro data is extremely challenging, since it requires multiple pharmacokinetic variables from one or more competing drugs to be known". The sentence is unclear for the reviewer.

For the reviewer, another drawback of in vitro studies is that clinically relevant substrates are not extensively studied in in vivo studies.

Example parameters have been added to the sentence to make it clearer and the reviewers suggested drawback has been added to the text. Some text has also been removed to shorten the introduction slightly.

"Extensive in vitro studies have been carried out to identify transporter-mediated DDIs [14]. However, accurately predicting the severity of clinically-relevant DDIs from in vitro data is extremely challenging and ideally requires knowledge of multiple parameters, including transporter expression levels, relative affinities of transporter for co-administered drugs, mechanism of inhibition, relative extents of drug-plasma protein binding and unbound drug concentrations [14, 15]. In addition, the clinically relevant substrates studied in vitro are not extensively studied in in vivo studies. Classically, pharmacokinetic (PK) information is derived from analyses of blood, urine and faeces samples, however such analyses are often complex and time consuming. Although in vitro data may be used to simulate plasma and liver concentration time profiles using a physiologically-based PK modelling approach [16], such an approach has limited predictive power for transporter-related interactions [17, 18]." (page 4).

4. "More recently Ulloa et al, using semi-quantitative and quantitative analyses, demonstrated a reduction in the washout time constant of gadoxetate in rats following co-administration of estradiol-17 β Dglucuronide and a dose-dependent reduction in the uptake rate and efflux kinetics (V_{max} , K_m) following co-administration of a chemokine antagonist"

Can the authors explain the wash out time? Wash out of liver concentrations over time?

The washout is the time constant associated with washout of concentration of gadoxetate in the liver. We have revised the sentence accordingly.

We have also included reference to a recent study by Giraudeau here (page 5).

5. "In this study we present the first use of our novel dual-input two-compartment uptake and efflux model, first used in humans [23], to monitor the transporter-mediated processes involved in the uptake and efflux of gadoxetate in mouse liver."

Since the model was previously published, the present study is not the first use. The authors apply the model previously developed in volunteers to DDI. The model is "based" on a dual-input two compartment uptake and efflux model.

We have reworded to clarify that this is the first preclinical application of the model (page 5).

6. Previous studies also investigated the mechanisms of rifampicin inhibition through hepatocyte transporters. Some of them included a pharmacokinetic modelling that should be commented.

This paper's focus is primarily on the technique, rather than the inhibitor rifampicin but we have included some of the reviewers suggested references.

"Rifampicin {Zheng, 2009} was chosen as the inhibitor for this study since it is used clinically and has previously been demonstrated to inhibit hepatic transporter-mediated uptake and efflux of gadobenate dimeglumine in rats {Daali, 2013} and the PET tracers [11C]rosuvastatin {He, 2014} and [11C]dehydropravastatin {Shingaki, 2013} rats and (15R)-11C-TIC-Me {Takashima, 2012} in humans." (page 5).

7. Overall, the introduction should be more concise and must include previous publications.

Some text has been removed from the introduction to compensate for the text added by making the changes suggested by the reviewer. We believe the introduction is as concise as possible.

Methods

1. What are "procedures 1 and 2 of PPL40/3268",

The procedure numbers are unnecessary and have been removed. The text now reads as follows:

"Animal experiments were performed according to the European ethical guidelines of animal experimentation, and in compliance with the UK Home Office Animals (Scientific Procedures) Act 1986, after review by the local ethics committee." (page 7).

2. "Inhibition experiments (Group B and C) were used to investigate the impact of in vivo DDIs at the OATP level, on gadoxetate pharmacokinetics in mouse liver."

As stated later by the authors, rifampicin also inhibits MRP2 and may activate the MRP3 efflux of gadobenate (Evidence of drug-drug interactions through uptake and efflux transport systems in rat hepatocytes: implications for cellular concentrations of competing drugs. Daali Y. <https://www.ncbi.nlm.nih.gov/pubmed/23708009>).

"at the OATP level" has been removed to simplify the meaning of the sentence (page 8).

Results

1. "The effect of rifampicin co-administration on gadoxetate-enhanced liver and blood concentration time series is shown in Figure 3." Blood should be replaced by portal.

Replaced, as suggested (page 12).

2. "The reduced uptake of gadoxetate into the liver is the likely cause of increased blood levels of gadoxetate even 70 minutes post-contrast agent administration". No statistics are presented for portal and liver concentrations between the 3 groups. Was it impossible to show portal and liver concentrations over time in the 3 groups in Fig. 3?

Figures 3 a and b, respectively, do show the liver and portal concentrations over time for each of the 3 groups. We have now compared the average portal concentration from 60 to 70 s between groups and reported the statistical significance.

"There is a statistically significant increase (one-way ANOVA $p < 0.0001$) in the average portal concentration (measured between 60 and 70 minutes post-contrast agent administration) for increasing rifampicin concentration. The reduced uptake of gadoxetate into the liver is the likely cause of these increased portal levels of gadoxetate." (page 12).

Discussion

1. "The uptake and efflux parameters therefore provide a measure of the transport of gadoxetate by a combination of transporters in vivo." Gadoxetate concentrations reflect the transport of the contrast agent through a combination of transporters.

We have removed "in vivo" from this sentence (page 14).

2. "Using the DCE technique allowed the derivation of quantitative information about the degree of reduction in the rate of active transport of gadoxetate, information that enhances the qualitative (?) data obtained from the concentration-time profiles." The reviewer does not understand while liver concentrations over time are qualitative data. This sentence appears several times in the manuscript.

The sentence has been changed to "Using the DCE technique allows the derivation of quantitative information about the degree of reduction in the rate of active transport of gadoxetate." (page 16).

3. The reviewer is not sure that 30 min was necessary for rifampicin to bind to OATP. In perfused rat livers, rifampicin sinusoidal clearance into hepatocytes is seen 5 min after the start of the perfusion

(of note, no protein was added in the perfusion solution). Moreover, RIF enters into hepatocytes and rifampicin concentrations into hepatocytes are high after 15 min. Thus, RIF can inhibit MRP2. However, its bile excretion is minor. Rifampicin metabolites were not measured. Interestingly, rifampicin exits from rat hepatocytes mainly back to sinusoids activating BOPTA efflux back. (Evidence of drug-drug interactions through uptake and efflux transport systems in rat hepatocytes: implications for cellular concentrations of competing drugs. Daali Y. <https://www.ncbi.nlm.nih.gov/pubmed/23708009>)

A 30 minute delay was suggested in Reference 27 (Kato et al 2002), although not for rifampicin in particular. The authors tested a series of clinical drugs (including rifampicin) and then injected the contrast agent 30 minutes after, possibly for consistency. Furthermore, practically the 30 minute gap gave as time to transfer the mouse, place it in the bore of the magnet, replace the catheter with that with the saline bolus and gadoxetate syringe, acquire localiser images, perform acquisitions for T1 measurement and then run the DCE sequence. We have added the following text to page 16.

"Rifampicin was administered 30 minutes prior to gadoxetate bolus administration, as suggested in Kato et al [27]. This was to allow for the drug to bind to the transporter proteins and produce an inhibitory effect, but also to allow the experimental procedures to be undertaken in a consistent and reproducible manner. However, inhibition alone may require less time, as described in Daali et al 2013 [31]."

4. "Drug-drug interaction at the sinusoidal site inhibited the transport of gadoxetate into the hepatocytes, which caused an increase in plasma gadoxetate level." The reviewer finds these findings interesting, but no statistics are presented.

This has now been addressed using the results of the statistical test described in the results section, comment 2 (page 12).

5. Maximal liver concentrations of gadoxetate are decreased in the presence of rifampicin, showing rifampicin inhibition of gadoxetate transport across OATP, but what about inhibition at the canalicular site? Because gadoxetate concentrations remain elevated in the liver over time, rifampicin is likely to inhibit the canalicular efflux. In light with the importance of drug concentrations for efficacy and safety, results on liver concentrations might respond to the presence of DDI before any compartmental modelling.

The decrease in maximal liver concentration of gadoxetate shows that OATP transport is the rate-limiting step. Our modelling also demonstrates a decreased in efflux with increased rifampicin concentration, which we believe is due to MRP2 inhibition. The initial liver concentrations are determined by OATP membrane transport only, whereas, over time, inhibition at the canalicular membrane also plays a role in determining liver concentrations. The extended concentration-time series data that we model is over a period of 70 mins, therefore our modelling can take into account both of these processes. However, if inhibition of canalicular efflux were the rate limiting step this would result in an increase in the concentration of gadoxetate, because it is not being pumped out of the hepatocyte. Our studies report both doses (20 mg/kg and 40 mg/kg) of rifampicin significantly reduce the observed liver gadoxetate concentration (Figure 3), which we interpret as rifampicin-mediated inhibition of gadoxetate uptake into the liver.

The reviewer feels both liver concentrations (in portal veins and livers) and clearance parameters should be related. Hepatocyte membrane transports determine cellular concentrations.

We agree that membrane transport is the major determinant of cellular concentrations for many compounds, including gadoxetate. Our model takes into account both hepatic clearance processes (i.e. OATP transport, the rate-limiting step) and portal/liver concentrations.

Table 1

The authors might correct Parameters and add the number of mice in each group.

Parameter pluralised in table 1. The total number of mice has been added to the table caption.

Tables 2 and 3

The authors might replace tracer by gadoxetate. Parameters. Healthy volunteers?

Replaced “tracer” with “gadoxetate”. Parameter pluralised in table 3. Replaced “healthy volunteers” with “control group”.

Fig. 3

Colours of points for groups 20 and 40 mg/kg are too similar to be distinguish on the graph.

The colours have been changed.

Reviewer #2:

From my perspective as a clinical radiologist, I would recommend to not only discuss possible implications of these finding on pharmacokinetic studies, but also on clinical routine liver MRI. Quantification of hepatic gadoxetate accumulation is developing into a non-invasive tool for the assessment of liver function and into a diagnostic tool for parenchymal liver disease. For example, it has been shown that gadoxetate liver MRI can help in the differential diagnosis and staging of liver fibrosis and cirrhosis, assess liver functional reserve of patients before undergoing liver surgery, help to diagnose and grade portal hypertension, estimate the survival probability of liver transplants, and many other clinical implications. The significance of pharmacologic OATP blockade that may alter clinical measurements of hepatic gadoxetate uptake might be an important bias for the above mentioned applications. Radiologists and clinicians should be made aware of this potential pitfall.

This is an important point. We have added the following text to the discussion. “Given its current diagnostic utility for the assessment of liver function and parenchymal liver disease, the significance of pharmacologic OATP blockade, which may alter clinical measurements of hepatic gadoxetate uptake, may be an important bias that radiologists and clinicians should consider.” (page 18).

Modelling gadoxetate liver uptake and efflux using DCE-MRI enables preclinical quantification of transporter drug-drug interactions.

Short title: Transporter DDIs quantified using DCE-MRI

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Conflicts of interest and sources of funding

The study was funded by a BBSRC and AstraZeneca CASE award. The authors declare no conflict of interest.

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Abstract

Objectives: To model the in vivo transporter-mediated uptake and efflux of the hepatobiliary contrast agent, gadoxetate, in the liver. The efficacy of the proposed technique was assessed for its ability to provide quantitative insights into drug-drug interactions (DDIs), using rifampicin as inhibitor.

Materials and Methods: Three groups of *C57* mice were scanned twice with a gadoxetate-enhanced dynamic Magnetic Resonance Imaging (DCE-MRI) protocol, using a 3-dimensional spoiled gradient-echo sequence for approximately 72 minutes. Before the second MRI session, two of the groups received a rifampicin dose of 20 (n=7) or 40 (n=7) mg/kg, respectively. Data from regions of interest in the liver were analysed using two simplifications of a two-compartment uptake and efflux model to provide estimates for the gadoxetate uptake rate (k_i) into the hepatocytes and its efflux rate (k_{ef}) into the bile. Both models were assessed for goodness-of-fit in the group without rifampicin (n=9) and the appropriate model was selected for assessing the ability to monitor DDIs in vivo.

Results: Seven out of nine mice from the group without rifampicin were assessed for model implementation and reproducibility. A simple three parameter model (k_i , k_{ef} , and extracellular space, v_{ecs}) adequately described the observed liver concentration time series with mean $k_i = 0.47 \pm 0.11 \text{ min}^{-1}$ and mean $k_{ef} = 0.039 \pm 0.016 \text{ min}^{-1}$. Visually the area under the liver concentration time profile was reduced for the groups receiving rifampicin. Furthermore, tracer kinetic modelling demonstrated a significant dose-dependent decrease in the uptake (5.9 and 17.3 fold decrease for 20mg/kg and 40mg/kg, respectively) and efflux rates (2.2 and 7.9 fold decrease) compared with the first scan for each group.

Conclusions: This study presents the first in vivo implementation of a two-compartment uptake and efflux model to monitor DDIs at the transporter-protein level, using the clinically-relevant OATP inhibitor, rifampicin. The technique has the potential to be a novel alternative to other methods, allowing real-time changes in transporter DDIs to be measured directly in vivo.

Introduction

Drug-drug interactions (DDIs) at the transporter protein level refer to the interaction between two or more drugs at the binding sites of membrane transporters, which may result in inhibition or enhancement of the pharmacological action of the administered compounds [1, 2]. Membrane transporters are located in various cell types and are responsible for the active transport of a wide range of endogenous and exogenous substances across the hydrophobic lipid bilayer. The liver, which plays a major role in drug metabolism and excretion, possesses a whole array of uptake and efflux transporters and is a major site of DDIs at the transporter protein level [3, 4]. It is known that interactions of drugs with membrane transporters can result in altered drug concentrations in tissues and/or blood, which in turn may have implications for both safety and efficacy [2, 5]. The influence of the organic anion transporting polypeptide (OATP) family members on pharmacokinetics has previously been reported [6]. Of particular importance in human liver are the OATP1B1, OATP1B3 and OATP2B1 transporters expressed on the sinusoidal membrane of hepatocytes, which facilitate the uptake of their drug substrate into hepatocytes and can be a determining factor in hepatic clearance [7]. Following substrate uptake into hepatocytes, members of the ATP-binding Cassette (ABC) superfamily of transporters expressed in the canalicular membrane, including the multidrug resistance-associated protein 2 (ABCC2, MRP2) and the multidrug resistance protein 1 (ABCB1, MDR1, P-glycoprotein), may be involved in the efflux of drugs and their metabolites from the hepatocyte into the bile [6] [8] [9] [10]

Hepatic clearance refers to the ability of the liver to extract a drug from blood, and studies have suggested that transporter-mediated drug uptake at the sinusoidal membrane of hepatocytes can be the rate-limiting step in hepatic clearance for some drugs [11-13].

Transporter-mediated DDIs in the liver therefore have the potential to significantly alter the pharmacokinetic profile of administered drugs and, if drug levels are altered, may impact

drug efficacy (if concentrations at the site of action are not adequate for effect) and/or drug safety (if concentrations are too high, leading to potential toxicity).

Extensive in vitro studies have been carried out to identify transporter-mediated DDIs [14]. However, accurately predicting the severity of clinically-relevant DDIs from in vitro data is extremely challenging and ideally requires knowledge of multiple parameters, including transporter expression levels, relative affinities of transporter for co-administered drugs, mechanism of inhibition, relative extents of drug-plasma protein binding and unbound drug concentrations [14, 15]. In addition, the clinically relevant substrates studied in vitro are not extensively studied in in vivo studies. Classically, pharmacokinetic (PK) information is derived from analyses of blood, urine and faeces samples, however such analyses are often complex and time consuming. Although in vitro data may be used to simulate plasma and liver concentration time profiles using a physiologically-based PK modelling approach [16], such an approach has limited predictive power for transporter-related interactions [17, 18].

Dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI) is a widely used technique that can provide quantitative insights into physiological processes taking place in vivo, by monitoring the real-time distribution of a bolus of contrast agent within the body. Specific hepatobiliary contrast agents include mangafodipir trisodium (Mn-DPDP), gadobenate dimeglumine (Gd-BOPTA), and gadoxetate (Gd-EOB-DTPA) [19]. Previous studies have demonstrated that gadoxetate (also known as Primovist, Eovist and gadoxetic acid), can potentially be used to monitor hepatic transporter-mediated processes in the liver [20-24]. Gadoxetate has been shown to be a substrate of OATP1B1, OATP1B3 and NTCP [25] uptake transporters and of the MRP2 and MRP3 efflux transporters [8]. Various in vitro studies have demonstrated the inhibitory effect of gadoxetate on the uptake of bromosulphophthalein (BSP) into OATP1B1 and OATP1B3 transfected HEK cells [25], as well as the inhibition of gadoxetate uptake in Oatp1 cRNA injected *Xenopus laevis* oocytes

following co-administration of BSP, taurocholate (TC), rifamycin SV and rifampicin (rifampin) [26]. Inhibition of gadoxetate uptake into the liver has also been shown indirectly in pre-clinical experiments with rats, where rifampicin co-administration caused a reduction in the liver enhancement time series compared to controls [27]. Ulloa et al, using semi-quantitative and quantitative analyses, demonstrated a reduction in the time constant associated with the washout in rat liver, following co-administration of estradiol-17 β D-glucuronide and a dose-dependent reduction in the uptake rate and efflux kinetics ($V_{\max}$, K_m) following co-administration of a chemokine antagonist [22, 28]. More recently, Giraudeau et al [29] used a compartmental modelling approach to quantify altered gadoxetate kinetics in a rat model of advanced liver fibrosis.

In this study we present the first preclinical application of our novel dual-input two-compartment uptake and efflux model, first used in humans [23], to monitor the transporter-mediated processes involved in the uptake and efflux of gadoxetate in mouse liver. Although murine OATPs differ slightly from human homologues (OATP1a/b in mouse) substrate affinities are considered to be similar. Rifampicin [30] was chosen as the inhibitor for this study since it is used clinically and has previously been demonstrated to inhibit hepatic transporter-mediated uptake and efflux of gadobenate dimeglumine in rats [31] and the PET tracers [11C]rosuvastatin [32] and [11C]dehydropravastatin [33] in rats and (15R)-11C-TIC-Me [34] in humans. This preclinical study allowed us to measure uptake/efflux of gadoxetate in the absence and presence of a clinically-relevant inhibitor rifampicin [30], thereby demonstrating the efficacy of our tracer kinetic model and its ability to provide non-invasive quantitative insights into DDIs at the transporter protein level.

Methods

Tracer Kinetic Models

Single-input two compartment uptake and efflux model (2CUE model)

The tracer kinetic model used (Figure 1) is based on the dual-input two compartment uptake and efflux model described and derived in full in [23]. A dual input approach is commonly used in the analysis of human DCE liver data (ie accounting for both arterial input function (AIF) and venous input function (VIF)). In the mouse the AIF and VIF signal time profiles exhibit similar temporal characteristics [35], with negligible delay and dispersion of the VIF compared to the AIF. Since the AIF is more likely to be compromised by inflow effects, a single input $C_I(t)$ derived from the hepatic portal vein ($c_v(t)$) was used, as defined in Equation 1:

$$C_I(t) = F_p \cdot \left\{ \frac{E_i}{1 - T_e/T_i} \cdot e^{-t/T_i} + \left(1 - \frac{E_i}{1 - T_e/T_i} \right) \cdot e^{-t/T_e} \right\} * c_v(t) / (1 - Hct) \quad [1]$$

where F_p is the total plasma flow, E_i ($k_i/[k_i + F_p]$) is the hepatic uptake fraction, k_i is the uptake rate into hepatocytes, T_e ($v_{ecs}/[F_p + k_i]$) is the extracellular mean transit time, v_{ecs} is the extracellular volume fraction, T_i (v_i/k_{ef}) is the intracellular mean transit times, v_i is the intracellular volume fraction and k_{ef} is the efflux rate out of the hepatocytes. In order to derive a separate estimate of k_{ef} , the intracellular volume fraction is required. This was assumed to be $1 - v_{ecs}$, since cell types other than hepatocytes represent a negligible proportion of the liver intracellular space [36]. The haematocrit level (Hct) was assumed to be 0.45 [37, 38].

Single-input two compartment uptake and efflux model with negligible mean extracellular transit time, T_e (2CUE $_{T_e \rightarrow 0}$)

An approximation to the 2CUE model may be obtained in the limit where the extracellular mean transit time, T_e , is negligible (or similarly plasma flow $\rightarrow \infty$). F_p has a broadening effect on the input, i.e. a low flow value would cause large dispersion of the input. In situations

where temporal resolution is low and/or the flow is high, any dispersion of the input would be very small causing the measured flow to appear infinite [39]. In these cases, the extracellular concentration in the liver is in equilibrium with the input concentration, and hence the former can be used as the input to the intracellular compartment. Using this approximation Equation 1 reduces to:

$$C_l(t) = [v_e \cdot \delta(t) + k_i \cdot e^{-t/T_i}] * c_v(t) / (1 - Hct) \quad [2]$$

where $\delta(t)$ is the delta function.

Animals and experimental procedures

DCE-MR Imaging protocol

All experiments were performed using a 7T preclinical MR scanner (Bruker BioSpin MRI). The MR sequence employed for both T_1 quantification and dynamic acquisition was the modified driven equilibrium Fourier transform (MDEFT) sequence. For T_1 quantification TR/TE=5.94/1.42 ms, FA=1°, 3°, 7°, 11°, 15° and 21°, FOV=3.4×3.4 cm², matrix size=128×128, number of slices=14, slice thickness=2 mm to cover the whole liver volume. The dynamic acquisition had the same TR/TE and FOV, and a flip angle of 12°. The temporal resolution of the dynamic sequence was 8.64 s.

Experimental procedures

Animal experiments were performed according to the European ethical guidelines of animal experimentation, and in compliance with the UK Home Office Animals (Scientific Procedures) Act 1986, after review by the local ethics committee. All mice were scanned twice (one week apart) following the above DCE-MRI protocol, with gadoxetate alone at session 1. Mice were scanned in three groups; Group A received gadoxetate alone at session 2 and served as the vehicle group (n=9, 22 weeks old male C57, 30-35 g, Charles River, UK),

and Groups B and C mice also followed the DCE-MRI protocol in session 2 with co-administration of rifampicin at a dose of 20 mg/kg (n=7, 25 weeks old male C57, 31-35 g, The Jackson Laboratory, UK) or 40 mg/kg (n=7, 25 weeks old male C57, 34-40 g, Charles River, UK), respectively. Inhibition experiments (Group B and C) were used to investigate the impact of in vivo DDIs on gadoxetate pharmacokinetics in mouse liver. The antibiotic rifampicin (mainly used in the treatment of tuberculosis; clinical dose: 10 mg/kg [40]) is a well-established inhibitor of human and rodent OATP transporters) [41]. Its interaction with gadoxetate has been reported in both in vitro and in vivo studies. [26, 27].

All mice were anaesthetized via 3% inhaled isoflurane in a gaseous mixture (O₂/air/N₂O) and maintained (at 1–2%) during the scanning process. The tail vein was cannulated using a 27-gauge (1/2 inch length) cannula secured in place using tape. The animals were transferred into the magnet's bore and body temperature was monitored and maintained using a hot air pump. Breathing rate was also monitored.

The variable flip angle images were acquired before contrast bolus administration for T_1 measurement, followed by the dynamic sequence. Approximately two minutes after the start of the dynamic acquisition, a bolus of gadoxetate at a dose of 25 μ mol/kg was injected into the lateral tail vein. The total scanning time was approximately 72 minutes and the total anaesthesia time did not exceed 90 minutes. At the end of session 1, mice were recovered from the anaesthesia using oxygen and a heated recovery box) and kept for a week prior to next session.

For Group A session 2, mice were scanned following the same procedures as session 1. For Groups B and C session 2, mice followed the same animal handling and imaging procedures described for session 1, but 30 minutes prior to administration of gadoxetate, animals were intravenously injected with the appropriate rifampicin dose using an automated injector. At

the end of the experiment all mice were terminated by overdose of anaesthesia (under Procedure 2 of PPL40/3268), confirmed by Schedule 1 method.

Postprocessing

Data were processed in Matlab (R2014a, MathWorks, Natick, MA, USA) and ImageJ. No motion correction was performed. Regions-of-interest (ROIs) were defined to cover the tissues of interest (liver and hepatic portal vein) and used to generate the corresponding T_1 estimates by fitting the variable flip angle data. The same ROIs were also applied to extract signal intensity time series from the dynamic images. These were converted to concentration series using the baseline T_1 estimates and assuming a R_1 relaxivity of gadoxetate of $4.8 \text{ mM}^{-1} \text{ s}^{-1}$ at 7 T. [42] This process of converting MR signal into concentration has been described in detail previously [20].

Model and Reproducibility Study

The two variations of the 2CUE model were fitted to the dynamic data (using the least squares fitting algorithm from Matlab's optimisation toolbox) obtained from Group A and the Akaike information criterion (AIC) was used to determine the most appropriate model for the data. The goodness-of-fit and optimisation performance was assessed using Akaike probabilities [43] (p_a) and by examining whether the generated parameters are independent of the initial input and within the applied constraints ($F_p < 10 \text{ ml/min/ml}$, $v_{ecs} < 0.5 \text{ ml/ml}$, $k_i < 10 \text{ min}^{-1}$, $k_{ef} < 10 \text{ min}^{-1}$). For the Akaike probabilities the number of dynamic points and the number of free parameters were used, which was either four or three for the full and simplified version of the 2CUE tracer kinetic model, respectively. A threshold of $p_a < 0.5$ for the Akaike probability was defined in favour of the simpler model to best describe the data. Following this initial implementation of both models, data fitted with the most appropriate model were used to assess the reproducibility of the parameter estimates, by calculating the

differences between the parameters in each session. These were used to calculate the reproducibility of the parameter estimates at the group (95% confidence intervals) and individual level (repeatability) [44, 45].

Inhibitor Studies

Based on the results of the initial implementation of the models, the appropriate model was used to fit the liver concentration time series of vehicle (Group A) and both inhibitor groups (Groups B and C) for both sessions. Changes in the parameter estimates within each group following the second session of the DCE-MRI experiment were assessed with a paired t-test.

Results

Model and Reproducibility Study

The final study cohort included eight mice in each session for Group A. Two mice were excluded from the analysis (one from each session) as a result of unsuccessful contrast administration. All successful scans were used for model comparison. Baseline T_1 relaxation times in the liver and VIF for Group A are shown in Table 1. Respiratory motion artefacts prevented the characterisation of the VIF signal in 6 out of the 16 scans. For this reason a population average input function was derived from those scans where it was possible to extract a VIF ($n=10$) and used as the input to the tracer kinetic model for all mice. Liver concentration time series were fitted using the proposed models (2CUE: F_p , v_{ecs} , k_i , k_{ef} , 2CUE_{Te→0}: v_{ecs} , k_i , k_{ef}).

Table 2 summarises the results of both tracer kinetic models. F_p and v_{ecs} estimates using the 2CUE model showed large variations and the results were dependent on the initial estimates used in the optimisation. Furthermore, in half of the cases v_{ecs} estimates reached the upper boundary limit. In the absence of the imposed constraints ($v_{ecs} < 0.5$ ml/ml) v_{ecs} estimates were converging towards non-physiological values (> 1 ml/ml). On the other hand, the simplified

form of the model ($2\text{CUE}_{T_e \rightarrow 0}$) generated more robust v_{ecs} estimates, with less variation and well within the constraints applied.

Example of model fits using both models are illustrated in Figure 2. By visual inspection both models provide good fits to the data. Figure 2a demonstrates an example of a 2CUE model fit that generated an estimate of v_{ecs} well within the constraints. However, the estimated Akaike probability suggests that the extra complexity of 2CUE is not justified over the use of the simplified form with negligible T_e ($p_a < 0.5$) (Figure 2b). Figure 2c illustrates an example of the 2CUE model v_{ecs} estimate reaching the upper boundary limit with an Akaike probability larger than 0.5. In fact, for half of the data, the full form of the 2CUE model generated Akaike probabilities larger than 0.5, however in all these cases v_{ecs} reached the upper boundary limit. Based on the fact that $2\text{CUE}_{T_e \rightarrow 0}$ generated parameter estimates independent of the initial input and the fact that Akaike probabilities were favouring the full form of 2CUE model only in cases where v_{ecs} estimates reached the upper limit (Figure 2c and d), the $2\text{CUE}_{T_e \rightarrow 0}$ was selected as the most suitable tracer kinetic model given the limitations of the acquisition technique used (e.g. low temporal resolution, noise levels).

A total of seven Group A mice successfully scanned on both sessions were used in the reproducibility assessment, which is summarised in Table 3. All parameter differences were tested for normality (Shapiro-Wilk test, $p > 0.05$) and for dependence of absolute value of the difference against the mean value of the two sessions (Kendall τ , $p > 0.05$). The most reproducible parameter was the uptake rate (k_i) in the hepatocytes and the least reproducible parameter was the efflux rate (k_{ef}) from the hepatocytes (within-subject coefficient of variation = 0.23, 0.14, 0.29 for v_{ecs} , k_i and k_{ef} , respectively). Repeatability and 95% confidence interval for the mean difference illustrate the magnitude of deviation for individual estimates or a group average that would allow assessment of a significant change following repetitive measurements, at the individual or group level, respectively.

Inhibitor Studies

For the 20 mg/kg rifampicin group (Group B) all seven mice were successfully scanned in both sessions. For the 40 mg/kg rifampicin group (Group C), all seven mice were successfully scanned in session 1 but two had to be excluded from session 2 due to unsuccessful injections which resulted in delivery of less than the full dose of rifampicin. For Group B, VIFs were successfully obtained in 4/7 mice for session 1 and all seven mice for session 2. For Group C, VIFs were successfully obtained in 6/7 mice for session 1 and all five mice for session 2. These VIFs were averaged to produce population VIFs for each group and each session. The variable flip angle data were used for baseline T_1 estimation of liver tissue and VIF. No significant differences were found in the T_1 estimates between session 1 and session 2 at the 95% significance level (Table 1).

The effect of rifampicin co-administration on gadoxetate-enhanced liver and portal concentration time series is shown in Figure 3. The inhibitor caused a decline in the wash in rate, a reduction in the peak concentration reached and a drop in the wash out rate compared to the controls. **There is a statistically significant increase (one-way ANOVA $p < 0.0001$) in the average portal concentration (measured between 60 and 70 minutes post-contrast agent administration) for increasing rifampicin concentration.** The reduced uptake of gadoxetate into the liver is the likely cause of these increased portal levels of gadoxetate.

Examples of fitted data for vehicle (Group A) and inhibitor groups (B and C), for both sessions are illustrated in Figure 4. Summary statistics are shown in Table 1 and the fold change in the mean estimates for all parameters are illustrated in Figure 5. The results demonstrate there is a significant reduction in both the gadoxetate uptake and efflux rates in hepatocytes of mice treated with 20 mg/kg or 40 mg/kg rifampicin, compared to vehicle treated controls (paired t-test, $p < 0.01$). Furthermore, there were significant differences in k_1

and k_{ef} parameter estimates between session 2 of the 20 mg/kg and 40 mg/kg rifampicin groups, which suggests a dose-dependent reduction in the uptake (two sampled t-test, $p=0.0015$) and efflux rates (two sampled t-test, $p=0.0068$) due to rifampicin co-administration. Significant differences were also found between session 1 parameter estimates of Group A versus Group B and Group A versus Group C, (two sampled t-test, $p < 0.05$) but not between groups B and C.

Discussion

Model and Reproducibility Study

Two approximations to the dual-input two-compartment uptake and efflux model [23] were implemented to describe the pharmacokinetics of gadoxetate in mouse liver. In the first, and in contrast to humans, a single input derived from the VIF was used since, at the temporal resolution of the DCE experiment in the present study, the AIF and VIF exhibited similar temporal characteristics, i.e. negligible dispersion or delay compared with the AIF [35]. The VIF was chosen as the input because the T_1 estimates and signal intensity time series in the AIF suggested that arterial blood was susceptible to inflow effects. The supply to the liver via the hepatic portal vein accounts for approximately 75% of the total blood. In the second, the additional approximation of negligible extracellular mean transit time was made.

Although both models were able to describe the liver time-series data, the full form of the single input 2CUE model did not always obtain v_{ecs} estimates within the imposed physiological range and was not robust to the starting parameters. F_p parameter also showed large variation within the group, indicating the inability of the model to accurately quantify both of these parameters in a mouse model, given the temporal resolution of the dynamic scan. Based on the mean F_p , v_{ecs} and k_i estimates, the extracellular mean transit time (T_e) is estimated to be less than 8 s. Given that this is lower than the temporal resolution used, it is

unsurprising that attempting to fit this model results in large variations in F_p and v_{ecs} estimates. In contrast, the $2CUE_{Te \rightarrow 0}$ model assumes a negligible extracellular mean transit time and is able to obtain more robust v_{ecs} parameter estimates.

The reproducibility assessment was based on the $2CUE_{Te \rightarrow 0}$ model, which best describes the data acquired. The results obtained provide a description of the transport of gadoxetate into and out of the mouse hepatocytes. The uptake rate reflects the transport of gadoxetate via specific transporters, such as OATPs and NTCP, expressed on the sinusoidal membrane of hepatocytes [8, 25]. Efflux rates describe the active transport of gadoxetate out of hepatocytes across the canalicular membrane and into the bile ducts by efflux transporters, including MRP2 [8] [46]. It is assumed here that any efflux back into the blood via sinusoidal transporters such as MRP3 and MRP4 is negligible. The substrate specificity of other canalicular efflux transporter candidates (eg. BCRP, P-gp) towards gadoxetate is yet to be investigated [8, 22]. The uptake and efflux parameters therefore provide a measure of the transport of gadoxetate by a combination of transporters.

Although the uptake rate exhibits low variability, the efflux rate k_{ef} showed lower reproducibility. This could be due to several factors, for example individual variability in intracellular binding of gadexotate or differing levels of competing endogenous substrates/inhibitors within the hepatocytes. Despite this observed variability in the efflux rates at the individual level, the results clearly demonstrate the sensitivity of DCE-MRI techniques to monitor changes in transporter-mediated processes. The 95% CI suggest that a group change of 16% and 30% in the mean uptake and efflux rate, respectively, would be significant. Changes of this magnitude could potentially occur with certain transporter genetic variants with reduced activity [47, 48], suggesting that DCE-MRI techniques could prove useful in determining the impact of pharmacogenomic variability on in vivo DDIs in the liver.

Inhibitor Studies

The experiments described involved a series of control scans in session 1, which serve as a baseline for the functional activity of the liver for each mouse. In session 2, rifampicin, a well-established inhibitor of both OATP and MRP2 [49] [50], was administered in Groups B (20 mg/kg dose) and C (40 mg/kg dose) prior to DCE-MRI analyses to examine whether DDIs at the transporter protein level can be monitored using the proposed DCE-MRI techniques. It is important to note that the clinically prescribed dose is 10 mg/kg, but a study suggested that the effect of a therapeutic dose of rifampicin in gadoxetate liver-time series would be very small [26] due to the high binding affinity of the drug to plasma proteins, i.e. approximately 80% bound, resulting in 20% free drug available to interact with hepatic transporters [51, 52]. For this reason we have used higher doses of rifampicin to study the principle of a rifampicin/gadoxetate DDI. It is, however, worth noting that the extent of plasma protein binding of therapeutic drugs varies across a wide range of values, with for example some drugs demonstrating low binding and higher levels of free drug. The binding affinity may also differ between humans and rodents.

Visual assessment of liver concentration–time profiles reveal large changes between session 1 and session 2 of inhibitor groups. Rifampicin was administered 30 minutes prior to gadoxetate bolus administration, as suggested in Kato et al [27]. This was to allow for the drug to bind to the transporter proteins and produce an inhibitory effect, but also to allow the experimental procedures to be undertaken in a consistent and reproducible manner. However, inhibition alone may require less time , as described in Daali et al 2013 [31]. Drug-drug interaction at the sinusoidal site inhibited the transport of gadoxetate into the hepatocytes, which caused an increase in plasma gadoxetate level. The wash in and wash out phases in the

liver concentration time series were visibly compromised at both doses, compared to their control groups.

Using the DCE technique allows the derivation of quantitative information about the degree of reduction in the rate of active transport of gadoxetate. The gadoxetate uptake rates were significantly compromised, with 5.9 and 17.3-fold decreases in the mean uptake rate ($P < 0.01$) following 20 mg/kg and 40 mg/kg rifampicin, respectively, compared to the corresponding control mean uptake rates. The mean efflux rates exhibited 2.2 and 7.9-fold decreases ($P < 0.01$), respectively, compared to the control scans. Moreover, the reduction in both uptake and efflux rates was dose-dependent ($P < 0.05$). The very large changes observed in this study give some confidence that a similar technique would be sensitive to inhibition of uptake at a more clinically relevant dose.

Limitation of this study include; age differences, the use of different suppliers and the fact that the mice were not controlled for fasting prior to the studies. These factors may cause experimental variations due to intrinsic physiological differences between mice. Despite these variations, the study was designed such that each individual mouse had a control scan (i.e. session 1, no rifampicin) and statistical tests for the effect of rifampicin were performed for the same mice under different conditions rather than between groups of mice. There were no differences in parameter estimates for the control scans (session 1) between the groups B and C (see Table 1). Therefore, the detected dose-dependent change is likely to be due to the effect of rifampicin, rather than any physiological differences between the groups.

Motion-induced artefacts precluded the use of individual VIF and this is of particular importance when an inhibitor is co-administered since the potency of inhibition might differ between mice, and hence an individually acquired VIF would partly account for this. Furthermore, noise in the acquired time series might have reduced the sensitivity of the

experiment. In addition, contrast-to-noise ratio is lower in the experiments with the inhibitor because of the reduced enhancement. Triggering was avoided because it would further compromise the temporal resolution of the dynamic series and post-processing image registration was not performed due to technical limitations. We also acknowledge the fact that transporter-mediated processes might be governed by non-linear kinetics, e.g. Michaelis-Menten kinetics, and the rate of uptake or efflux might saturate at high concentrations of gadoxetate either in the sinusoidal site of the hepatocytes or within the hepatocyte cells. As described previously, due to the maximum limit in the number of identifiable parameters in a two-compartment tracer kinetic model [53], the absence of an explicit solution to models that incorporate non-linear kinetics and the limitations imposed by acquisition requirements, linear approximations for both uptake and efflux processes were assumed [23, 54].

Implications

The results of this study highlight the potential of hepatobiliary contrast agents such as gadoxetate to be used as a tool to investigate DDIs in the liver, *in vivo*. DCE-MRI with gadoxetate offers the opportunity to monitor the disposition of the tracer *in vivo* without the need for regular sampling of blood, urine and faeces and provides direct, real-time information from tissues of interest (e.g. liver, kidneys) that can be used to improve and refine pharmacokinetic models. This becomes particularly helpful when modelling hepatobiliary transport since it provides easy access to additional information, such as biliary excretions. Studying interactions at the level of OATP transporters with clinically relevant drugs can be difficult, particularly if a drug is a potent OATP inhibitor, and may lead to drug-induced toxicity for compounds that are substrates of OATPs. However, preclinical mouse models can certainly supplement such studies - although the murine homologues OATP1a/1b

do differ slightly from human OATP1B1/1B3/2B1, they share both sequence similarities (e.g. OATP1B1 and OATP1b2 have 65% amino acid identity [55]) and substrates and demonstrate similar hepatic uptake PK [56]. The use of humanized OATP mouse models also shows promise. [2, 57]. Thus DCE-MRI with gadoxetate offers the opportunity to monitor the disposition of the tracer in vivo and provides direct information from tissues of interest (e.g. liver, kidneys) that can be used to improve and refine pharmacokinetic models.

Gadoxetate is routinely used in liver imaging in clinical practice and is considered safe for patients without renal impairment [58, 59]. However, in March 2017 the European Medicines Agency (EMA) recommended the suspension of the marketing authorisations for four linear gadolinium based contrast agents due to evidence that small amounts of gadolinium may be deposited in the brain [60]. Gadoxetate is also a linear agent and whilst it was not one of the agents recommended for suspension due to its important diagnostic utility, the extent to which this may affect its use in clinical DDI studies is uncertain. **Given its current diagnostic utility for the assessment of liver function and parenchymal liver disease, the significance of pharmacologic OATP blockade, which may alter clinical measurements of hepatic gadoxetate uptake, may be an important bias that radiologists and clinicians should consider.**

Conclusion

In conclusion, this study reports a novel tracer kinetic model that assesses liver function in terms of both the uptake and efflux of gadoxetate alone or in the presence of a transporter inhibitor. The model was implemented in mouse models, where the reproducibility of the technique was studied and subsequently tested for its ability to detect and quantify in vivo DDIs at the transporter-protein level, using the clinically-relevant OATP inhibitor rifampicin.

Where there is uncertainty in the level of inhibition expected from the co-administration of drugs in humans, this technique may potentially provide an alternative and safer tool to investigate DDIs in vivo.

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Figure Legends

Figure 1. Schematic representation of a single-input two compartment uptake and efflux model (2CUE).

Figure 2. Example of fits using the 2CUE and 2CUE_{Te→0} models on two mice. (a) & (b) demonstrate an example where Akaike probabilities suggest the use of 2CUE_{Te→0} ($p_a = 0.29$). (c) & (d) illustrate an example where Akaike probabilities suggest that 2CUE is more appropriate for the dataset ($p_a = 0.92$), however the v_{ecs} estimate for 2CUE model (red box) reached the upper boundary constraints imposed in the optimisation process.

Figure 3. Mean liver and hepatic portal vein concentration time series of gadoxetate obtained from the session 1 and 2 of vehicle, and session 2 of 20 mg/kg and 40 mg/kg of rifampicin groups.

Figure 4. Examples of tracer-kinetic model fits for individual mice from vehicle (Group A) and inhibitor groups (B and C). (a, c, e) represents session 1 where DCE-MRI was performed with gadoxetate alone and (b, d, f) represents the corresponding session 2 where mice received the appropriate rifampicin dose (0, 20, 40 mg/kg) according to their group, prior to the DCE-MRI experiment with gadoxetate.

Figure 5. Box plots showing the fold-change in the parameter estimates between session 1 and 2 of each group (denotes* a significant change following paired t-test $p < 0.01$ and ** a significant change following a two-sample t-test $p < 0.05$).

Parameters	2CUE	2CUE _{Te→0}
	Mean ± σ	Mean ± σ
F_p (ml/min/ml)	2.01 ± 0.66	NA
v_{ecs} (ml/ml)	0.34 ± 0.17	0.31 ± 0.09
k_i (/min)	0.61 ± 0.16	0.47 ± 0.11
k_{ef} (/min)	0.035 ± 0.014	0.039 ± 0.016

Table 1. Parameter estimate mean values (± standard deviation) for 2CUE and 2CUE_{Te→0} across all 8 mice for both sessions.

Parameters	Control Group (n=7)			20 mg/kg Rifampicin Group (n=7)			40 mg/kg Rifampicin Group (n=5)		
	Session 1	Session 2	p-value	Session 1	Session 2	p-value	Session 1	Session 2	p-value
	Mean ± σ	Mean ± σ		Mean ± σ	Mean ± σ		Mean ± σ	Mean ± σ	
v_{ecs} (ml/ml)	0.32 ± 0.10	0.30 ± 0.09	0.97	0.20 ± 0.03	0.17 ± 0.04	0.11	0.20 ± 0.03	0.19 ± 0.02	0.55
k_i (/min)	0.43 ± 0.10	0.50 ± 0.12	0.22	0.56 ± 0.11	0.08 ± 0.03	< 0.01*	0.53 ± 0.07	0.03 ± 0.01	< 0.01*
k_{ef} (/min)	0.034 ± 0.012	0.044 ± 0.019	0.13	0.070 ± 0.027	0.021 ± 0.001	< 0.01*	0.052 ± 0.009	0.006 ± 0.003	< 0.01*
T_l Liver (s)	1.00 ± 0.11	0.98 ± 0.12	0.43	1.06 ± 0.13	1.03 ± 0.10	0.98	1.04 ± 0.12	1.08 ± 0.80	0.52
T_l VIF (s)	2.04 ± 0.21	1.97 ± 0.29	0.28	2.07 ± 0.14	2.15 ± 0.15	0.18	2.12 ± 0.23	1.03 ± 0.10	0.73

Table 2. Estimated **gadoxetate** kinetic parameters for mice scanned in both sessions for control, 20 mg/kg and 40 mg/kg groups (*denotes a significant difference between session 1 and 2).

Parameters	Mean $\pm \sigma$	Mean Difference	95% CI for the mean difference ($\pm$)	Repeatability
v_{ecs} (ml/ml)	0.30 ± 0.09	0.03	0.07	0.19
k_i (/min)	0.45 ± 0.11	0.07	0.07	0.18
k_{ef} (/min)	0.039 ± 0.017	-0.009	0.012	0.031

Table 3. Estimated tracer kinetic parameters and reproducibility statistics for $2\text{CUE}_{\text{Te} \rightarrow 0}$. CI, confidence interval for the control group. Repeatability is the value of the 95% limit for the difference between two measurements on an individual.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Abstract

Objectives: To model the in vivo transporter-mediated uptake and efflux of the hepatobiliary contrast agent, gadoxetate, in the liver. The efficacy of the proposed technique was assessed for its ability to provide quantitative insights into drug-drug interactions (DDIs), using rifampicin as inhibitor.

Materials and Methods: Three groups of *C57* mice were scanned twice with a gadoxetate-enhanced dynamic Magnetic Resonance Imaging (DCE-MRI) protocol, using a 3-dimensional spoiled gradient-echo sequence for approximately 72 minutes. Before the second MRI session, two of the groups received a rifampicin dose of 20 (n=7) or 40 (n=7) mg/kg, respectively. Data from regions of interest in the liver were analysed using two simplifications of a two-compartment uptake and efflux model to provide estimates for the gadoxetate uptake rate (k_i) into the hepatocytes and its efflux rate (k_{ef}) into the bile. Both models were assessed for goodness-of-fit in the group without rifampicin (n=9) and the appropriate model was selected for assessing the ability to monitor DDIs in vivo.

Results: Seven out of nine mice from the group without rifampicin were assessed for model implementation and reproducibility. A simple three parameter model (k_i , k_{ef} , and extracellular space, v_{ecs}) adequately described the observed liver concentration time series with mean $k_i = 0.47 \pm 0.11 \text{ min}^{-1}$ and mean $k_{ef} = 0.039 \pm 0.016 \text{ min}^{-1}$. Visually the area under the liver concentration time profile was reduced for the groups receiving rifampicin. Furthermore, tracer kinetic modelling demonstrated a significant dose-dependent decrease in the uptake (5.9 and 17.3 fold decrease for 20mg/kg and 40mg/kg, respectively) and efflux rates (2.2 and 7.9 fold decrease) compared with the first scan for each group.

Conclusions: This study presents the first in vivo implementation of a two-compartment uptake and efflux model to monitor DDIs at the transporter-protein level, using the clinically-relevant OATP inhibitor, rifampicin. The technique has the potential to be a novel alternative to other methods, allowing real-time changes in transporter DDIs to be measured directly in vivo.

Introduction

Drug-drug interactions (DDIs) at the transporter protein level refer to the interaction between two or more drugs at the binding sites of membrane transporters, which may result in inhibition or enhancement of the pharmacological action of the administered compounds [1, 2]. Membrane transporters are located in various cell types and are responsible for the active transport of a wide range of endogenous and exogenous substances across the hydrophobic lipid bilayer. The liver, which plays a major role in drug metabolism and excretion, possesses a whole array of uptake and efflux transporters and is a major site of DDIs at the transporter protein level [3, 4]. It is known that interactions of drugs with membrane transporters can result in altered drug concentrations in tissues and/or blood, which in turn may have implications for both safety and efficacy [2, 5]. The influence of the organic anion transporting polypeptide (OATP) family members on pharmacokinetics has previously been reported [6]. Of particular importance in human liver are the OATP1B1, OATP1B3 and OATP2B1 transporters expressed on the sinusoidal membrane of hepatocytes, which facilitate the uptake of their drug substrate into hepatocytes and can be a determining factor in hepatic clearance [7]. Following substrate uptake into hepatocytes, members of the ATP-binding Cassette (ABC) superfamily of transporters expressed in the canalicular membrane, including the multidrug resistance-associated protein 2 (ABCC2, MRP2) and the multidrug resistance protein 1 (ABCB1, MDR1, P-glycoprotein), may be involved in the efflux of drugs and their metabolites from the hepatocyte into the bile [6] [8] [9] [10]

Hepatic clearance refers to the ability of the liver to extract a drug from blood, and studies have suggested that transporter-mediated drug uptake at the sinusoidal membrane of hepatocytes can be the rate-limiting step in hepatic clearance for some drugs [11-13]. Transporter-mediated DDIs in the liver therefore have the potential to significantly alter the pharmacokinetic profile of administered drugs and, if drug levels are altered, may impact

drug efficacy (if concentrations at the site of action are not adequate for effect) and/or drug safety (if concentrations are too high, leading to potential toxicity).

Extensive *in vitro* studies have been carried out to identify transporter-mediated DDIs [14]. However, accurately predicting the severity of clinically-relevant DDIs from *in vitro* data is extremely challenging and ideally requires knowledge of multiple parameters, including transporter expression levels, relative affinities of transporter for co-administered drugs, mechanism of inhibition, relative extents of drug-plasma protein binding and unbound drug concentrations [14, 15]. In addition, the clinically relevant substrates studied *in vitro* are not extensively studied in *in vivo* studies. Classically, pharmacokinetic (PK) information is derived from analyses of blood, urine and faeces samples, however such analyses are often complex and time consuming. Although *in vitro* data may be used to simulate plasma and liver concentration time profiles using a physiologically-based PK modelling approach [16], such an approach has limited predictive power for transporter-related interactions [17, 18].

Dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI) is a widely used technique that can provide quantitative insights into physiological processes taking place *in vivo*, by monitoring the real-time distribution of a bolus of contrast agent within the body. Specific hepatobiliary contrast agents include mangafodipir trisodium (Mn-DPDP), gadobenate dimeglumine (Gd-BOPTA), and gadoxetate (Gd-EOB-DTPA) [19]. Previous studies have demonstrated that gadoxetate (also known as Primovist, Eovist and gadoxetic acid), can potentially be used to monitor hepatic transporter-mediated processes in the liver [20-24]. Gadoxetate has been shown to be a substrate of OATP1B1, OATP1B3 and NTCP [25] uptake transporters and of the MRP2 and MRP3 efflux transporters [8]. Various *in vitro* studies have demonstrated the inhibitory effect of gadoxetate on the uptake of bromosulphophthalein (BSP) into OATP1B1 and OATP1B3 transfected HEK cells [25], as well as the inhibition of gadoxetate uptake in Oatp1 cRNA injected *Xenopus laevis* oocytes

following co-administration of BSP, taurocholate (TC), rifamycin SV and rifampicin (rifampin) [26]. Inhibition of gadoxetate uptake into the liver has also been shown indirectly in pre-clinical experiments with rats, where rifampicin co-administration caused a reduction in the liver enhancement time series compared to controls [27]. Ulloa et al, using semi-quantitative and quantitative analyses, demonstrated a reduction in the time constant associated with the washout in rat liver, following co-administration of estradiol-17 β D-glucuronide and a dose-dependent reduction in the uptake rate and efflux kinetics ($V_{\max}$, K_m) following co-administration of a chemokine antagonist [22, 28]. More recently, Giraudeau et al [29] used a compartmental modelling approach to quantify altered gadoxetate kinetics in a rat model of advanced liver fibrosis.

In this study we present the first preclinical application of our novel dual-input two-compartment uptake and efflux model, first used in humans [23], to monitor the transporter-mediated processes involved in the uptake and efflux of gadoxetate in mouse liver. Although murine OATPs differ slightly from human homologues (OATP1a/b in mouse) substrate affinities are considered to be similar. Rifampicin [30] was chosen as the inhibitor for this study since it is used clinically and has previously been demonstrated to inhibit hepatic transporter-mediated uptake and efflux of gadobenate dimeglumine in rats [31] and the PET tracers [11C]rosuvastatin [32] and [11C]dehydropravastatin [33] in rats and (15R)-11C-TIC-Me [34] in humans. This preclinical study allowed us to measure uptake/efflux of gadoxetate in the absence and presence of a clinically-relevant inhibitor rifampicin [30], thereby demonstrating the efficacy of our tracer kinetic model and its ability to provide non-invasive quantitative insights into DDIs at the transporter protein level.

Methods

Tracer Kinetic Models

Single-input two compartment uptake and efflux model (2CUE model)

The tracer kinetic model used (Figure 1) is based on the dual-input two compartment uptake and efflux model described and derived in full in [23]. A dual input approach is commonly used in the analysis of human DCE liver data (ie accounting for both arterial input function (AIF) and venous input function (VIF)). In the mouse the AIF and VIF signal time profiles exhibit similar temporal characteristics [35], with negligible delay and dispersion of the VIF compared to the AIF. Since the AIF is more likely to be compromised by inflow effects, a single input $C_l(t)$ derived from the hepatic portal vein ($c_v(t)$) was used, as defined in Equation 1:

$$C_l(t) = F_p \cdot \left\{ \frac{E_i}{1 - T_e/T_i} \cdot e^{-t/T_i} + \left(1 - \frac{E_i}{1 - T_e/T_i} \right) \cdot e^{-t/T_e} \right\} * c_v(t) / (1 - Hct) \quad [1]$$

where F_p is the total plasma flow, E_i ($k_i/[k_i + F_p]$) is the hepatic uptake fraction, k_i is the uptake rate into hepatocytes, T_e ($v_{ecs}/[F_p + k_i]$) is the extracellular mean transit time, v_{ecs} is the extracellular volume fraction, T_i (v_i/k_{ef}) is the intracellular mean transit times, v_i is the intracellular volume fraction and k_{ef} is the efflux rate out of the hepatocytes. In order to derive a separate estimate of k_{ef} , the intracellular volume fraction is required. This was assumed to be $1 - v_{ecs}$, since cell types other than hepatocytes represent a negligible proportion of the liver intracellular space [36]. The haematocrit level (Hct) was assumed to be 0.45 [37, 38].

Single-input two compartment uptake and efflux model with negligible mean extracellular transit time, T_e (2CUE $_{T_e \rightarrow 0}$)

An approximation to the 2CUE model may be obtained in the limit where the extracellular mean transit time, T_e , is negligible (or similarly plasma flow $\rightarrow \infty$). F_p has a broadening effect on the input, i.e. a low flow value would cause large dispersion of the input. In situations

where temporal resolution is low and/or the flow is high, any dispersion of the input would be very small causing the measured flow to appear infinite [39]. In these cases, the extracellular concentration in the liver is in equilibrium with the input concentration, and hence the former can be used as the input to the intracellular compartment. Using this approximation Equation 1 reduces to:

$$C_l(t) = [v_e \cdot \delta(t) + k_i \cdot e^{-t/T_i}] * c_v(t) / (1 - Hct) \quad [2]$$

where $\delta(t)$ is the delta function.

Animals and experimental procedures

DCE-MR Imaging protocol

All experiments were performed using a 7T preclinical MR scanner (Bruker BioSpin MRI). The MR sequence employed for both T_1 quantification and dynamic acquisition was the modified driven equilibrium Fourier transform (MDEFT) sequence. For T_1 quantification TR/TE=5.94/1.42 ms, FA=1°, 3°, 7°, 11°, 15° and 21°, FOV=3.4×3.4 cm², matrix size=128×128, number of slices=14, slice thickness=2 mm to cover the whole liver volume. The dynamic acquisition had the same TR/TE and FOV, and a flip angle of 12°. The temporal resolution of the dynamic sequence was 8.64 s.

Experimental procedures

Animal experiments were performed according to the European ethical guidelines of animal experimentation, and in compliance with the UK Home Office Animals (Scientific Procedures) Act 1986, after review by the local ethics committee. All mice were scanned twice (one week apart) following the above DCE-MRI protocol, with gadoxetate alone at session 1. Mice were scanned in three groups; Group A received gadoxetate alone at session 2 and served as the vehicle group (n=9, 22 weeks old male C57, 30-35 g, Charles River, UK),

and Groups B and C mice also followed the DCE-MRI protocol in session 2 with co-administration of rifampicin at a dose of 20 mg/kg (n=7, 25 weeks old male C57, 31-35 g, The Jackson Laboratory, UK) or 40 mg/kg (n=7, 25 weeks old male C57, 34-40 g, Charles River, UK), respectively. Inhibition experiments (Group B and C) were used to investigate the impact of in vivo DDIs on gadoxetate pharmacokinetics in mouse liver. The antibiotic rifampicin (mainly used in the treatment of tuberculosis; clinical dose: 10 mg/kg [40]) is a well-established inhibitor of human and rodent OATP transporters) [41]. Its interaction with gadoxetate has been reported in both in vitro and in vivo studies. [26, 27].

All mice were anaesthetized via 3% inhaled isoflurane in a gaseous mixture (O₂/air/N₂O) and maintained (at 1–2%) during the scanning process. The tail vein was cannulated using a 27-gauge (1/2 inch length) cannula secured in place using tape. The animals were transferred into the magnet's bore and body temperature was monitored and maintained using a hot air pump. Breathing rate was also monitored.

The variable flip angle images were acquired before contrast bolus administration for T_1 measurement, followed by the dynamic sequence. Approximately two minutes after the start of the dynamic acquisition, a bolus of gadoxetate at a dose of 25 μ mol/kg was injected into the lateral tail vein. The total scanning time was approximately 72 minutes and the total anaesthesia time did not exceed 90 minutes. At the end of session 1, mice were recovered from the anaesthesia using oxygen and a heated recovery box) and kept for a week prior to next session.

For Group A session 2, mice were scanned following the same procedures as session 1. For Groups B and C session 2, mice followed the same animal handling and imaging procedures described for session 1, but 30 minutes prior to administration of gadoxetate, animals were intravenously injected with the appropriate rifampicin dose using an automated injector. At

the end of the experiment all mice were terminated by overdose of anaesthesia (under Procedure 2 of PPL40/3268), confirmed by Schedule 1 method.

Postprocessing

Data were processed in Matlab (R2014a, MathWorks, Natick, MA, USA) and ImageJ. No motion correction was performed. Regions-of-interest (ROIs) were defined to cover the tissues of interest (liver and hepatic portal vein) and used to generate the corresponding T_1 estimates by fitting the variable flip angle data. The same ROIs were also applied to extract signal intensity time series from the dynamic images. These were converted to concentration series using the baseline T_1 estimates and assuming a R_1 relaxivity of gadoxetate of $4.8 \text{ mM}^{-1} \text{ s}^{-1}$ at 7 T. [42] This process of converting MR signal into concentration has been described in detail previously [20].

Model and Reproducibility Study

The two variations of the 2CUE model were fitted to the dynamic data (using the least squares fitting algorithm from Matlab's optimisation toolbox) obtained from Group A and the Akaike information criterion (AIC) was used to determine the most appropriate model for the data. The goodness-of-fit and optimisation performance was assessed using Akaike probabilities [43] (p_a) and by examining whether the generated parameters are independent of the initial input and within the applied constraints ($F_p < 10 \text{ ml/min/ml}$, $v_{\text{ecs}} < 0.5 \text{ ml/ml}$, $k_i < 10 \text{ min}^{-1}$, $k_{\text{ef}} < 10 \text{ min}^{-1}$). For the Akaike probabilities the number of dynamic points and the number of free parameters were used, which was either four or three for the full and simplified version of the 2CUE tracer kinetic model, respectively. A threshold of $p_a < 0.5$ for the Akaike probability was defined in favour of the simpler model to best describe the data. Following this initial implementation of both models, data fitted with the most appropriate model were used to assess the reproducibility of the parameter estimates, by calculating the

differences between the parameters in each session. These were used to calculate the reproducibility of the parameter estimates at the group (95% confidence intervals) and individual level (repeatability) [44, 45].

Inhibitor Studies

Based on the results of the initial implementation of the models, the appropriate model was used to fit the liver concentration time series of vehicle (Group A) and both inhibitor groups (Groups B and C) for both sessions. Changes in the parameter estimates within each group following the second session of the DCE-MRI experiment were assessed with a paired t-test.

Results

Model and Reproducibility Study

The final study cohort included eight mice in each session for Group A. Two mice were excluded from the analysis (one from each session) as a result of unsuccessful contrast administration. All successful scans were used for model comparison. Baseline T_1 relaxation times in the liver and VIF for Group A are shown in Table 1. Respiratory motion artefacts prevented the characterisation of the VIF signal in 6 out of the 16 scans. For this reason a population average input function was derived from those scans where it was possible to extract a VIF ($n=10$) and used as the input to the tracer kinetic model for all mice. Liver concentration time series were fitted using the proposed models (2CUE: F_p , v_{ecs} , k_i , k_{ef} , 2CUE_{Te→0}: v_{ecs} , k_i , k_{ef}).

Table 2 summarises the results of both tracer kinetic models. F_p and v_{ecs} estimates using the 2CUE model showed large variations and the results were dependent on the initial estimates used in the optimisation. Furthermore, in half of the cases v_{ecs} estimates reached the upper boundary limit. In the absence of the imposed constraints ($v_{ecs} < 0.5$ ml/ml) v_{ecs} estimates were converging towards non-physiological values (>1 ml/ml). On the other hand, the simplified

form of the model ($2\text{CUE}_{T_e \rightarrow 0}$) generated more robust v_{ecs} estimates, with less variation and well within the constraints applied.

Example of model fits using both models are illustrated in Figure 2. By visual inspection both models provide good fits to the data. Figure 2a demonstrates an example of a 2CUE model fit that generated an estimate of v_{ecs} well within the constraints. However, the estimated Akaike probability suggests that the extra complexity of 2CUE is not justified over the use of the simplified form with negligible T_e ($p_a < 0.5$) (Figure 2b). Figure 2c illustrates an example of the 2CUE model v_{ecs} estimate reaching the upper boundary limit with an Akaike probability larger than 0.5. In fact, for half of the data, the full form of the 2CUE model generated Akaike probabilities larger than 0.5, however in all these cases v_{ecs} reached the upper boundary limit. Based on the fact that $2\text{CUE}_{T_e \rightarrow 0}$ generated parameter estimates independent of the initial input and the fact that Akaike probabilities were favouring the full form of 2CUE model only in cases where v_{ecs} estimates reached the upper limit (Figure 2c and d), the $2\text{CUE}_{T_e \rightarrow 0}$ was selected as the most suitable tracer kinetic model given the limitations of the acquisition technique used (e.g. low temporal resolution, noise levels).

A total of seven Group A mice successfully scanned on both sessions were used in the reproducibility assessment, which is summarised in Table 3. All parameter differences were tested for normality (Shapiro-Wilk test, $p > 0.05$) and for dependence of absolute value of the difference against the mean value of the two sessions (Kendall τ , $p > 0.05$). The most reproducible parameter was the uptake rate (k_i) in the hepatocytes and the least reproducible parameter was the efflux rate (k_{ef}) from the hepatocytes (within-subject coefficient of variation = 0.23, 0.14, 0.29 for v_{ecs} , k_i and k_{ef} , respectively). Repeatability and 95% confidence interval for the mean difference illustrate the magnitude of deviation for individual estimates or a group average that would allow assessment of a significant change following repetitive measurements, at the individual or group level, respectively.

Inhibitor Studies

For the 20 mg/kg rifampicin group (Group B) all seven mice were successfully scanned in both sessions. For the 40 mg/kg rifampicin group (Group C), all seven mice were successfully scanned in session 1 but two had to be excluded from session 2 due to unsuccessful injections which resulted in delivery of less than the full dose of rifampicin. For Group B, VIFs were successfully obtained in 4/7 mice for session 1 and all seven mice for session 2. For Group C, VIFs were successfully obtained in 6/7 mice for session 1 and all five mice for session 2. These VIFs were averaged to produce population VIFs for each group and each session. The variable flip angle data were used for baseline T_1 estimation of liver tissue and VIF. No significant differences were found in the T_1 estimates between session 1 and session 2 at the 95% significance level (Table 1).

The effect of rifampicin co-administration on gadoxetate-enhanced liver and portal concentration time series is shown in Figure 3. The inhibitor caused a decline in the wash in rate, a reduction in the peak concentration reached and a drop in the wash out rate compared to the controls. There is a statistically significant increase (one-way ANOVA $p < 0.0001$) in the average portal concentration (measured between 60 and 70 minutes post-contrast agent administration) for increasing rifampicin concentration. The reduced uptake of gadoxetate into the liver is the likely cause of these increased portal levels of gadoxetate.

Examples of fitted data for vehicle (Group A) and inhibitor groups (B and C), for both sessions are illustrated in Figure 4. Summary statistics are shown in Table 1 and the fold change in the mean estimates for all parameters are illustrated in Figure 5. The results demonstrate there is a significant reduction in both the gadoxetate uptake and efflux rates in hepatocytes of mice treated with 20 mg/kg or 40 mg/kg rifampicin, compared to vehicle treated controls (paired t-test, $p < 0.01$). Furthermore, there were significant differences in k_1

and k_{ef} parameter estimates between session 2 of the 20 mg/kg and 40 mg/kg rifampicin groups, which suggests a dose-dependent reduction in the uptake (two sampled t-test, $p=0.0015$) and efflux rates (two sampled t-test, $p=0.0068$) due to rifampicin co-administration. Significant differences were also found between session 1 parameter estimates of Group A versus Group B and Group A versus Group C, (two sampled t-test, $p < 0.05$) but not between groups B and C.

Discussion

Model and Reproducibility Study

Two approximations to the dual-input two-compartment uptake and efflux model [23] were implemented to describe the pharmacokinetics of gadoxetate in mouse liver. In the first, and in contrast to humans, a single input derived from the VIF was used since, at the temporal resolution of the DCE experiment in the present study, the AIF and VIF exhibited similar temporal characteristics, i.e. negligible dispersion or delay compared with the AIF [35]. The VIF was chosen as the input because the T_1 estimates and signal intensity time series in the AIF suggested that arterial blood was susceptible to inflow effects. The supply to the liver via the hepatic portal vein accounts for approximately 75% of the total blood. In the second, the additional approximation of negligible extracellular mean transit time was made.

Although both models were able to describe the liver time-series data, the full form of the single input 2CUE model did not always obtain v_{ecs} estimates within the imposed physiological range and was not robust to the starting parameters. F_p parameter also showed large variation within the group, indicating the inability of the model to accurately quantify both of these parameters in a mouse model, given the temporal resolution of the dynamic scan. Based on the mean F_p , v_{ecs} and k_i estimates, the extracellular mean transit time (T_e) is estimated to be less than 8 s. Given that this is lower than the temporal resolution used, it is

unsurprising that attempting to fit this model results in large variations in F_p and v_{ecs} estimates. In contrast, the $2CUE_{Te \rightarrow 0}$ model assumes a negligible extracellular mean transit time and is able to obtain more robust v_{ecs} parameter estimates.

The reproducibility assessment was based on the $2CUE_{Te \rightarrow 0}$ model, which best describes the data acquired. The results obtained provide a description of the transport of gadoxetate into and out of the mouse hepatocytes. The uptake rate reflects the transport of gadoxetate via specific transporters, such as OATPs and NTCP, expressed on the sinusoidal membrane of hepatocytes [8, 25]. Efflux rates describe the active transport of gadoxetate out of hepatocytes across the canalicular membrane and into the bile ducts by efflux transporters, including MRP2 [8] [46]. It is assumed here that any efflux back into the blood via sinusoidal transporters such as MRP3 and MRP4 is negligible. The substrate specificity of other canalicular efflux transporter candidates (eg. BCRP, P-gp) towards gadoxetate is yet to be investigated [8, 22]. The uptake and efflux parameters therefore provide a measure of the transport of gadoxetate by a combination of transporters.

Although the uptake rate exhibits low variability, the efflux rate k_{ef} showed lower reproducibility. This could be due to several factors, for example individual variability in intracellular binding of gadexotate or differing levels of competing endogenous substrates/inhibitors within the hepatocytes. Despite this observed variability in the efflux rates at the individual level, the results clearly demonstrate the sensitivity of DCE-MRI techniques to monitor changes in transporter-mediated processes. The 95% CI suggest that a group change of 16% and 30% in the mean uptake and efflux rate, respectively, would be significant. Changes of this magnitude could potentially occur with certain transporter genetic variants with reduced activity [47, 48], suggesting that DCE-MRI techniques could prove useful in determining the impact of pharmacogenomic variability on in vivo DDIs in the liver.

Inhibitor Studies

The experiments described involved a series of control scans in session 1, which serve as a baseline for the functional activity of the liver for each mouse. In session 2, rifampicin, a well-established inhibitor of both OATP and MRP2 [49] [50], was administered in Groups B (20 mg/kg dose) and C (40 mg/kg dose) prior to DCE-MRI analyses to examine whether DDIs at the transporter protein level can be monitored using the proposed DCE-MRI techniques. It is important to note that the clinically prescribed dose is 10 mg/kg, but a study suggested that the effect of a therapeutic dose of rifampicin in gadoxetate liver-time series would be very small [26] due to the high binding affinity of the drug to plasma proteins, i.e. approximately 80% bound, resulting in 20% free drug available to interact with hepatic transporters [51, 52]. For this reason we have used higher doses of rifampicin to study the principle of a rifampicin/gadoxetate DDI. It is, however, worth noting that the extent of plasma protein binding of therapeutic drugs varies across a wide range of values, with for example some drugs demonstrating low binding and higher levels of free drug. The binding affinity may also differ between humans and rodents.

Visual assessment of liver concentration–time profiles reveal large changes between session 1 and session 2 of inhibitor groups. Rifampicin was administered 30 minutes prior to gadoxetate bolus administration, as suggested in Kato et al [27]. This was to allow for the drug to bind to the transporter proteins and produce an inhibitory effect, but also to allow the experimental procedures to be undertaken in a consistent and reproducible manner. However, inhibition alone may require less time, as described in Daali et al 2013 [31]. Drug-drug interaction at the sinusoidal site inhibited the transport of gadoxetate into the hepatocytes, which caused an increase in plasma gadoxetate level. The wash in and wash out phases in the

liver concentration time series were visibly compromised at both doses, compared to their control groups.

Using the DCE technique allows the derivation of quantitative information about the degree of reduction in the rate of active transport of gadoxetate. The gadoxetate uptake rates were significantly compromised, with 5.9 and 17.3-fold decreases in the mean uptake rate ($P < 0.01$) following 20 mg/kg and 40 mg/kg rifampicin, respectively, compared to the corresponding control mean uptake rates. The mean efflux rates exhibited 2.2 and 7.9-fold decreases ($P < 0.01$), respectively, compared to the control scans. Moreover, the reduction in both uptake and efflux rates was dose-dependent ($P < 0.05$). The very large changes observed in this study give some confidence that a similar technique would be sensitive to inhibition of uptake at a more clinically relevant dose.

Limitation of this study include; age differences, the use of different suppliers and the fact that the mice were not controlled for fasting prior to the studies. These factors may cause experimental variations due to intrinsic physiological differences between mice. Despite these variations, the study was designed such that each individual mouse had a control scan (i.e. session 1, no rifampicin) and statistical tests for the effect of rifampicin were performed for the same mice under different conditions rather than between groups of mice. There were no differences in parameter estimates for the control scans (session 1) between the groups B and C (see Table 1). Therefore, the detected dose-dependent change is likely to be due to the effect of rifampicin, rather than any physiological differences between the groups.

Motion-induced artefacts precluded the use of individual VIF and this is of particular importance when an inhibitor is co-administered since the potency of inhibition might differ between mice, and hence an individually acquired VIF would partly account for this. Furthermore, noise in the acquired time series might have reduced the sensitivity of the

experiment. In addition, contrast-to-noise ratio is lower in the experiments with the inhibitor because of the reduced enhancement. Triggering was avoided because it would further compromise the temporal resolution of the dynamic series and post-processing image registration was not performed due to technical limitations. We also acknowledge the fact that transporter-mediated processes might be governed by non-linear kinetics, e.g. Michaelis-Menten kinetics, and the rate of uptake or efflux might saturate at high concentrations of gadoxetate either in the sinusoidal site of the hepatocytes or within the hepatocyte cells. As described previously, due to the maximum limit in the number of identifiable parameters in a two-compartment tracer kinetic model [53], the absence of an explicit solution to models that incorporate non-linear kinetics and the limitations imposed by acquisition requirements, linear approximations for both uptake and efflux processes were assumed [23, 54].

Implications

The results of this study highlight the potential of hepatobiliary contrast agents such as gadoxetate to be used as a tool to investigate DDIs in the liver, *in vivo*. DCE-MRI with gadoxetate offers the opportunity to monitor the disposition of the tracer *in vivo* without the need for regular sampling of blood, urine and faeces and provides direct, real-time information from tissues of interest (e.g. liver, kidneys) that can be used to improve and refine pharmacokinetic models. This becomes particularly helpful when modelling hepatobiliary transport since it provides easy access to additional information, such as biliary excretions. Studying interactions at the level of OATP transporters with clinically relevant drugs can be difficult, particularly if a drug is a potent OATP inhibitor, and may lead to drug-induced toxicity for compounds that are substrates of OATPs. However, preclinical mouse models can certainly supplement such studies - although the murine homologues OATP1a/1b

do differ slightly from human OATP1B1/1B3/2B1, they share both sequence similarities (e.g. OATP1B1 and OATP1b2 have 65% amino acid identity [55]) and substrates and demonstrate similar hepatic uptake PK [56]. The use of humanized OATP mouse models also shows promise. [2, 57]. Thus DCE-MRI with gadoxetate offers the opportunity to monitor the disposition of the tracer in vivo and provides direct information from tissues of interest (e.g. liver, kidneys) that can be used to improve and refine pharmacokinetic models.

Gadoxetate is routinely used in liver imaging in clinical practice and is considered safe for patients without renal impairment [58, 59]. However, in March 2017 the European Medicines Agency (EMA) recommended the suspension of the marketing authorisations for four linear gadolinium based contrast agents due to evidence that small amounts of gadolinium may be deposited in the brain [60]. Gadoxetate is also a linear agent and whilst it was not one of the agents recommended for suspension due to its important diagnostic utility, the extent to which this may affect its use in clinical DDI studies is uncertain. Given its current diagnostic utility for the assessment of liver function and parenchymal liver disease, the significance of pharmacologic OATP blockade, which may alter clinical measurements of hepatic gadoxetate uptake, may be an important bias that radiologists and clinicians should consider.

Conclusion

In conclusion, this study reports a novel tracer kinetic model that assesses liver function in terms of both the uptake and efflux of gadoxetate alone or in the presence of a transporter inhibitor. The model was implemented in mouse models, where the reproducibility of the technique was studied and subsequently tested for its ability to detect and quantify in vivo DDIs at the transporter-protein level, using the clinically-relevant OATP inhibitor rifampicin.

Where there is uncertainty in the level of inhibition expected from the co-administration of drugs in humans, this technique may potentially provide an alternative and safer tool to investigate DDIs in vivo.

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Figure Legends

Figure 1. Schematic representation of a single-input two compartment uptake and efflux model (2CUE).

Figure 2. Example of fits using the 2CUE and 2CUE_{Te→0} models on two mice. (a) & (b) demonstrate an example where Akaike probabilities suggest the use of 2CUE_{Te→0} ($p_a = 0.29$). (c) & (d) illustrate an example where Akaike probabilities suggest that 2CUE is more appropriate for the dataset ($p_a = 0.92$), however the v_{ecs} estimate for 2CUE model (red box) reached the upper boundary constraints imposed in the optimisation process.

Figure 3. Mean liver and hepatic portal vein concentration time series of gadoxetate obtained from the session 1 and 2 of vehicle, and session 2 of 20 mg/kg and 40 mg/kg of rifampicin groups.

Figure 4. Examples of tracer-kinetic model fits for individual mice from vehicle (Group A) and inhibitor groups (B and C). (a, c, e) represents session 1 where DCE-MRI was performed with gadoxetate alone and (b, d, f) represents the corresponding session 2 where mice received the appropriate rifampicin dose (0, 20, 40 mg/kg) according to their group, prior to the DCE-MRI experiment with gadoxetate.

Figure 5. Box plots showing the fold-change in the parameter estimates between session 1 and 2 of each group (denotes* a significant change following paired t-test $p < 0.01$ and ** a significant change following a two-sample t-test $p < 0.05$).

Parameters	2CUE	2CUE _{Te→0}
	Mean ± σ	Mean ± σ
F_p (ml/min/ml)	2.01 ± 0.66	NA
v_{ecs} (ml/ml)	0.34 ± 0.17	0.31 ± 0.09
k_i (/min)	0.61 ± 0.16	0.47 ± 0.11
k_{ef} (/min)	0.035 ± 0.014	0.039 ± 0.016

Table 1. Parameter estimate mean values (± standard deviation) for 2CUE and 2CUE_{Te→0} across all 8 mice for both sessions.

Parameters	Control Group (n=7)			20 mg/kg Rifampicin Group (n=7)			40 mg/kg Rifampicin Group (n=5)		
	Session 1	Session 2	p-value	Session 1	Session 2	p-value	Session 1	Session 2	p-value
	Mean ± σ	Mean ± σ		Mean ± σ	Mean ± σ		Mean ± σ	Mean ± σ	
v_{ecs} (ml/ml)	0.32 ± 0.10	0.30 ± 0.09	0.97	0.20 ± 0.03	0.17 ± 0.04	0.11	0.20 ± 0.03	0.19 ± 0.02	0.55
k_i (/min)	0.43 ± 0.10	0.50 ± 0.12	0.22	0.56 ± 0.11	0.08 ± 0.03	< 0.01*	0.53 ± 0.07	0.03 ± 0.01	< 0.01*
k_{ef} (/min)	0.034 ± 0.012	0.044 ± 0.019	0.13	0.070 ± 0.027	0.021 ± 0.001	< 0.01*	0.052 ± 0.009	0.006 ± 0.003	< 0.01*
T_I Liver (s)	1.00 ± 0.11	0.98 ± 0.12	0.43	1.06 ± 0.13	1.03 ± 0.10	0.98	1.04 ± 0.12	1.08 ± 0.80	0.52
T_I VIF (s)	2.04 ± 0.21	1.97 ± 0.29	0.28	2.07 ± 0.14	2.15 ± 0.15	0.18	2.12 ± 0.23	1.03 ± 0.10	0.73

Table 2. Estimated gadoxetate kinetic parameters for mice scanned in both sessions for control, 20 mg/kg and 40 mg/kg groups (*denotes a significant difference between session 1 and 2).

Parameters	Mean $\pm \sigma$	Mean Difference	95% CI for the mean difference ($\pm$)	Repeatability
v_{ecs} (ml/ml)	0.30 ± 0.09	0.03	0.07	0.19
k_i (/min)	0.45 ± 0.11	0.07	0.07	0.18
k_{ef} (/min)	0.039 ± 0.017	-0.009	0.012	0.031

Table 3. Estimated tracer kinetic parameters and reproducibility statistics for $2\text{CUE}_{\text{Te} \rightarrow 0}$. CI, confidence interval for the control group. Repeatability is the value of the 95% limit for the difference between two measurements on an individual.