

Assessment of DCEMRI with gadoxetate as a biomarker of drug induced cholestasis

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INTRODUCTION

One of the major patterns of drug-induced liver injury (DILI) observed in man is cholestatic DILI. The mechanisms are complex and include decreased bile formation, which results in increased bile acid levels in blood [1]. Hepatobiliary transporters mediate uptake and excretion of drugs and endogenous substances. It has been proposed that inhibition of biliary efflux transporters may play a key role in initiating cholestatic DILI [2]. Previously, we have shown that Dynamic Contrast Enhanced MRI (DCEMRI) with gadoxetate can be used as a suitable biomarker of transient cholestatic DILI caused by the well characterised cholestatic agent estradiol- β -D-glucuronide in the rat *in vivo* [3]. However, its utility for characterising cholestatic effects of investigational drugs has not yet been established. We have used an investigational chemokine agonist (CKA) as a model compound that caused cholestatic DILI in rats at high doses. The CKA compound inhibits the hepatobiliary efflux transporters Bsep and Mrp2 *in vitro*. It is not yet clear whether it is also a substrate and/or inhibitor of Oatp1 which mediates the uptake of gadoxetate [4]. The aim of the present study is to assess the sensitivity of DCEMRI as a potential biomarker of drug-induced cholestasis by exploring the dose-dependent relationship between the CKA model compound, gadoxetate uptake and efflux kinetics, and clinical chemistry markers of DILI.

METHODS

30 male Hsd Han Wistar rats (298 \pm 26 g, Harlan, UK) were divided into 5 groups: Vehicle (aqueous 0.5% (w/v) hydroxypropyl-methylcellulose/0.1% (w/v) polysorbate 80), 2000, 500, 200 and 20 mg/kg of CKA. Rats were fasted overnight, dosed orally with CKA or vehicle, and anaesthetised using 3% isoflurane in air and maintained at 1.5%-2%. R1 maps of the liver and spleen were acquired using an Inversion Recovery FISP sequence (TR/TE=3.4/1.5ms, FA=4°, 128x128 acq. matrix, 10x2mm slices) and then DCEMRI data were acquired continuously for 1 hour with an IntraGate FLASH [6] sequence (TR/TE=60.3/1.4ms, FA=30°, 128x128 acq. matrix, FOV 60x60mm², 10x2mm slices, 60 time frames). 5 min after DCE data acquisition commenced and 60 min after administration of CKA compound or vehicle, 25 μ mol/kg of gadoxetate were administered *i.v.* via the tail vein. MRI was performed at 4.7T (Oxford Instrument magnet, Bruker Biospin Avance III console running ParaVision 5.1 and a 72mm quadrature birdcage volume transceiver). IntraGate FLASH images provided anatomical reference for the liver. After imaging, rats were euthanized with an overdose of isoflurane and blood samples were taken for measuring plasma chemistry parameters. Region of interests (RoI) covering the liver and spleen were manually selected using ImageJ (<http://rsb.info.nih.gov/ij/>) in both the IR-FISP and DCEMRI acquisitions, for calculating the mean longitudinal relaxation, R1 and time course extraction, respectively. Signal intensity was converted to concentration of contrast agent using the standard relationship (e.g. [7]) and assuming 5.15 s⁻¹mM⁻¹ the relaxivity of gadoxetate at 4.7T [8]. The concentration of gadoxetate in hepatocytes was calculated from the concentration in liver and spleen, assuming equal volume fraction of blood and extracellular space [9]. The uptake of gadoxetate into hepatocytes was characterised by the slope of the time course in the first 3 min after contrast injection. The biliary elimination was described by the efflux rate of the time course, modelled as a single exponential decay function [3]. Parameters estimation was done in Matlab (www.mathworks.com). The statistical significance of the effect of CKA compound on the kinetics of gadoxetate uptake and clearance were assessed with a non-parametric test for medians (Kruskal-Wallis) of the logarithm of the parameters (95% significance level).

RESULTS

Figure 1 shows representative images of 3 rats (vehicle, 500mg/kg and 200mg/kg CKA) at baseline and 5 time points after contrast injection (t=0, 6, 18, 30, 42 and 60 min). Figure 2A shows the concentration of gadoxetate in the hepatocytes, expressed as the median for each group (error bars representing the standard error). Note that for the 2000 and 500mg/kg groups the concentration of gadoxetate in the hepatocytes increased over the acquisition time and there was a lack of enhancement of bile reaching the intestines (Figures 1G to 1L). The elimination rate characterising the efflux was lower (p=0.003) in the 200mg/kg group compared to vehicle and 20mg/kg groups. Figure 2B shows the rate of uptake for each group. There were no differences between vehicle and 20mg/kg groups (p=0.34), and between 500mg/kg and 2000mg/kg groups (p=0.87), while the rate of uptake of the 200mg/kg group was lower (p=0.05) than vehicle and higher (p=0.01) than the 500 mg/kg group. In Figure 3, plasma bile acids (3A) and total bilirubin (3B) concentrations are presented. These were elevated in response to compound treatment, and the magnitude of changes in these plasma markers correlated inversely with the observed changes in gadoxetate uptake and excretion.

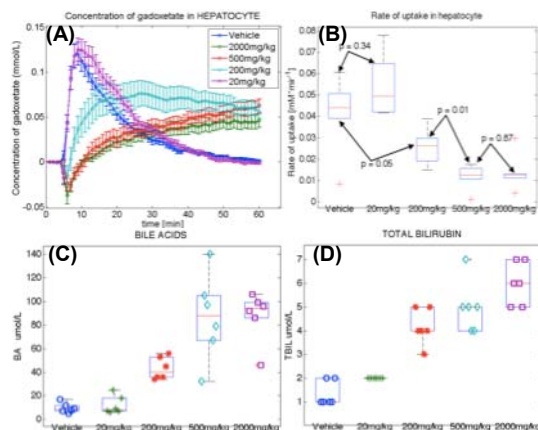


Figure 2: (A) Concentration of gadoxetate in hepatocytes. (B) Rate of uptake of gadoxetate into hepatocyte, first 3 min after contrast injection. Concentration of (C) bile acids and (D) total bilirubin in plasma at the end of the acquisition.

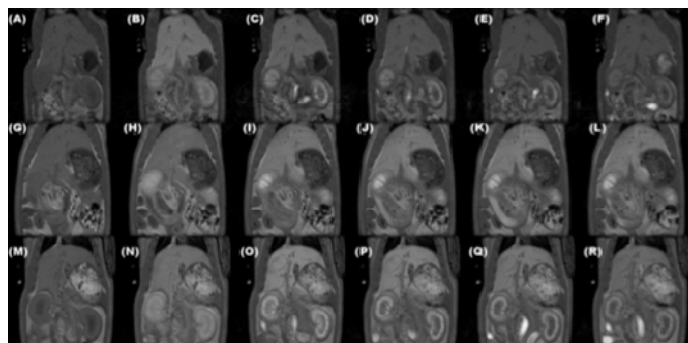


Figure 1: Examples of dynamic images, vehicle (top row), 500mg/kg (middle row) and 200mg/kg (bottom row) t=0(A;G;M), 6(B;H;N), 18(C;I;O), 30(D;J;P), 42(E;K;Q) and 60 (F;L;R) min after contrast injection.

DISCUSSION

In rats, oral dosing of the CKA compound causes cholestatic DILI and increases plasma bile acid and bilirubin levels, indicative of functional impairment of bile flow. The virtually complete inhibition of gadoxetate transport into bile at doses higher than 500mg/kg, partial inhibition at 200mg/kg, and absence of inhibition at 20mg/kg and in vehicle indicate *in vivo* inhibition of Mrp2 by CKA. The observed inhibition of gadoxetate uptake into hepatocytes by CKA also suggests that both compounds may be substrates of Oatp1. We conclude that gadoxetate DCEMRI can detect drug induced cholestasis, and has the potential to serve as a novel biomarker of cholestasis caused by candidate drugs. Furthermore, since gadoxetate transport is responsive to inhibition of the Oatp1 and Mrp2 hepatobiliary transporters and has been used clinically, it may be a sensitive and specific biomarker of transporter function that is applicable both in laboratory animals and in patients.

REFERENCES: [1] Holt et al. *The AAPS Journal*, 8(1):E48-E54, 2006. [2] Wagner et al. *J. Hepatol*, 51:565-580, 2009. [3] Ulloa et al. *ISMRM* 18:2593, 2010. [4] Tsuda et al. *ISMRM* 18:558, 2010. [6] Bishop et al. *MRM* 55(3):472-7, 2006. [7] Roberts et al. *JMRI* 23(4):554-63, 2006. [8] Rohrer et al. *Invest. Radiol.* 40(11):715-24, 2005. [9] Leinhard et al. *ISMRM* 17:2074, 2009