Effects of a single intravenous dose of Estradiol-17β D-glucuronide on biliary excretion: Assessment with gadoxetate DCEMRI

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Introduction

Mechanisms of drug-induced liver injury (DILI) are poorly understood and difficult to identify [1]. It has been proposed that inhibition of hepatobiliary transporters, including the bile salt export pump (Bsep) and multidrug resistance protein 2 (Mrp2) [2], which mediate hepatic uptake and excretion of numerous clinical drugs [3], could play a key role in initiation of cholestatic DILI. Cholestasis is the impairment of bile formation, one of the vital liver functions and one of the major patterns of liver injury observed in humans. It has been shown that a single bolus dose of Estradiol-17β D-glucuronide ($E_2$17G) induces dose-dependant acute but transient cholestasis in rats by affecting Mrp2 and Bsep [4,5]. Gadoxetate (Primovist, Bayer Schering) is a hepatobiliary-specific MRI contrast agent (CA) cleared by both kidney and liver [6] and has successfully been used to detect and characterise focal liver lesions [7]. In rats, Oatp1 mediates the fraction of gadoxetate taken up by the liver, while Mrp2 mediates its biliary excretion [8], suggesting that it can be used as a biomarker for cholestasis [9]. Despite an emerging interest in this area, relatively few studies [10,7] have reported parametric characterisation of liver uptake and clearance of gadoxetate. The aim of this work is to assess whether DCEMRI could detect a change in hepatic uptake of gadoxetate due to transient bile flow impairment following a single i.v. dose of $E_2$17G.

Methods

Data from six healthy male HanWistar rats (8-10 weeks, 250-300 grs, Harlan, UK) were analysed. Animals were anaesthetised using 3% isoflurane in air and maintained at 1.5%-2% throughout the imaging procedure. Rectal temperature and respiration rate were monitored and maintained at 35-38ºC, and 50-60 breaths/min, respectively. Three rats received a single i.v. dose of 21 μmol/kg $E_2$17G (Sigma, Poole, UK) and three were injected with vehicle (Saline/10% bovine serum albumin) 30 minutes before. i.v. administration via tail vein catheter of 250umol/kg gadoxetate. MRI was performed at 9.4T (Varian *Inova*, 63mm quadrature birdcage volume transceiver). Respiratory-gated coronal FLASH images (TR/TE=60/3.67ms, FA=50°, 256x128 matrix size, FOV 60x60mm^2, 8x2mm slices) provided anatomical reference for the liver. The same FLASH sequence, with no respiratory-gating was used to acquire dynamic axial T1W images continuously for up to 2 hours (7.68 sec/volume, 2 min baseline). After imaging, rats were euthanased with an overdose of isoflurane and blood samples were taken for measuring plasma chemistry parameters.

The time course of the signal intensity was analysed on a Region of Interest basis (RoI). RoIs covering the liver were defined by thresholding the maximum intensity projection along the time axis of the dynamic images using ImageJ (http://rsb.info.nih.gov/ij). The mean over the RoI was used to characterise the enhancement in the liver. The late phase of this time-dependant function characterises the biliary elimination of the contrast agent and was modelled in Matlab (http://www.mathworks.com) as a single-exponential decay: $\mathbf{NI(t>t_0)=A*exp(-(t-t_0)/\tau_1)}$, with NI the normalised signal intensity of the liver, t_0, the inflexion time where the contrast agent starts being cleared into bile (i.e. late hepatic phase, manually determined from the data) and τ_1 the elimination rate of the CA in the liver. The statistical significance of $E_2$17G induced effect on biliary elimination of gadoxetate, as determined by τ_1, was assessed with a one-sided two-samples t-test of the logarithm of τ_1 between groups.

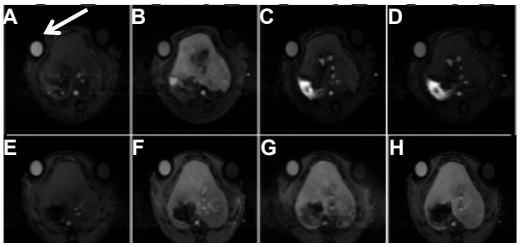

Figure 1: Examples of dynamic images, vehicle (top row) and treated (bottom row) t=0(A;E),10(B;F), 40(C;G),70(D;H) min after contrast injection. Signal intensity in the liver was normalised to the signal of the phantom (white arrow in (a)).

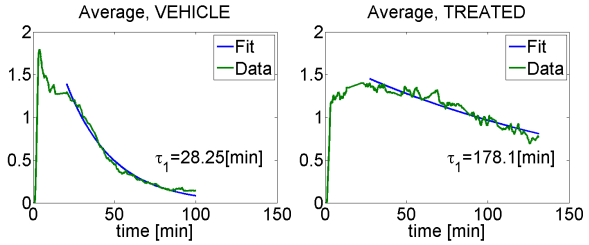

Figure 2: (Green line) Time course of the average signal intensity (normalised to the signal in the phantom) of the vehicle (left) and treated (right) groups. (Blue line) Output of the hepatic elimination model of the contrast agent.

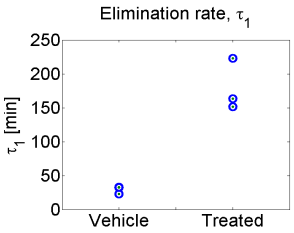

Figure 3: Values of τ_1 for each animal and each group. Note that in the vehicle group, 2 values were too similar each other that appear as one single point in the graph

Results

Fig. 1 shows representative images of a control and an $E_2$17G treated animal at baseline and three time points after contrast injection (t=0, 10, 40 and 70 min). Fig. 2 shows the average time course of the liver enhancement. Fig. 3 shows τ_1 for each group, values were significantly different (p=0.0002) and ranged from 22.9 to 33.2 min (vehicle) and from 151.4 to 222.8 min (treated). Table 1 summarises the mean values obtained for several plasma markers, treated animals show an increase in bilirubin (total and conjugated) and bile acids compared to vehicle, indicating functional inhibition of Mrp2 and Bsep, respectively. Although the number of samples is too small to establish a significant correlation between blood markers and τ_1 in each group, all of them were higher in the treated group, respect to the vehicle. Note that blood samples were taken at the end of the experiments, when signal had returned to normal in the vehicle group (Fig. 2), therefore a weaker correlation can be expected in this case, compared to the treated group.

	TBIL	BILD	ALP	AST	ALT	GDLH	BA	τ_1
Vehicle	2.0	0.0	105.0	156.0	95.5	40.0	13.0	28.3
Treated	11.3	5.7	126.0	824.0	827.0	423.0	68.0	178.1

Table 1: Average plasma chemistry pararmeters for each group.Total (TBIL) and conjugated (BILD) bilirubin, alkaline phosphatase (ALP), aspartate (AST) and alanine (ALT) aminotransferases, glutamate dehydrogenase (GLDH), bile acids (BA) and hepatic elimination rate (τ_1).

Discussion

We have shown that transient impairment of bile flow induced with a single i.v. dose of $E_2$17G can be characterised with gadoxetate DCEMRI. To the best of our knowledge this is the first *in-vivo* study relating transient cholestasis with characterisation of gadoxetate DCEMRI excretion. In contrast to existing methods for examining cholestasis, imaging is non-invasive and can therefore be used in the assessment of novel candidate drugs to investigate the effects on liver function and to study interactions of compound with hepatic transporters. This method has the potential to be an early biomarker for drug-induced liver injury. **References:** [1]The AAPS Journal 8(1); Article 6,2006.[2]Journal of Hepatology 51(3):565-580,2009.[3]Invest. Radiol. 37(12):680-4,2002.[4]JPET 307:306–313,2003.[5]Am J Physiol Gastrointest Liver Physiol 293: G391–G402,2007.[6]Invest. Radiol. 29(2):213-6,1994.[7]JMRI 29:1323–1331,2009.[8]ISMRM 2009, p4085.[9]Radiology 90(3):753-8,1994.[10]ESMRMB 2009, poster 88.