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 (E2;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 E2;17G.

Methods
Data from six healthy male HanWistar rats (8-10 weeks, 250-300 gns, 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 35-38°C, and 50-60 breaths/min, respectively. Three rats received a single i.v. dose of 21 μmol/kg E2;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 250μmol/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 60x60mm², 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 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).

Results
Fig. 1 shows representative images of a control and an E2;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 τ1 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 τ1 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.

Discussion