We conclude that the THLE-CYP cell model enables *in vitro* investigation of both CYP independent and CYP mediated processes which may contribute to DILI, and also exploration of underlying mechanisms (e.g. bioactivation of clozapine by 2D6). If used in drug discovery, this approach has the potential to aid in rational design and selection of compounds with reduced DILI liability, and could enable early deselection of drug candidates with significant potential to cause DILI in man.

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P26

Evaluation of the relationship between inhibition of the bile salt export pump *in vitro* and risk of drug-induced liver injury in man

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It has been proposed, that inhibition of hepatobiliary transporters may play an important role in the development of drug-induced liver injury (DILI) (Wagner, 2009; Stieger, 2007). To provide further understanding of the relationship between inhibition of the canalicular transporter bile salt efflux pump (Bsep; Abcb11) and DILI in man, we have assessed inhibition of Bsep activity *in vitro* by hepatotoxic and non-hepatotoxic marketed drugs.

Inhibition of rat Bsep was analysed in inverted plasma membrane vesicles prepared from transfected *Sf*21 insect cells. We quantified the adenosine triphosphate (ATP)-dependent uptake of the probe substrate [^{3}H]-taurocholate by a rapid filtration technique (Stieger et al., 2000). In addition, inhibition of Bsep function in sandwich-cultured primary rat hepatocytes was assessed using cholyllysylfluorescein (CLF) as a probe substrate, by quantifying CLF efflux into canalicular pockets using high content imaging and a Cellomics Arrayscan^{TM} algorithm (manuscript in preparation).

Potent inhibition of rat Bsep activity in vesicles (IC_{50} < 300 μM) was observed with 22/42 drugs (52%) associated with cholestatic or mixed liver injury in man, 6/25 drugs (32%) associated with hepatocellular liver injury and 5/23 non-hepatotoxic drugs (17%) (Fig. 1A).

A high correlation between potency of rat Bsep inhibition in vesicles and hepatocytes was observed (r^2 = 0.79). However, the apparent potency of inhibition was greater in hepatocytes than in vesicles for many of the tested drugs (Fig. 1B). The compounds that exhibited potent inhibition of Bsep in both experimental systems included the withdrawn drugs troglitazone and nefazodone, and bosentan and ketoconazole, both of which have a DILI black box warning.

We conclude that potent Bsep inhibition by drugs correlates with increased risk of cholestatic drug hepatotoxicity in man, and that *in vitro* evaluation of this liability is useful for DILI hazard identification.

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P27

Oxidative stress via hydrogen peroxide and Menadione does not induce the secretion of IGFBP-5 in primary rat hepatocytes

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Mortality rates are on the increase from liver disease in the developed world, particularly in Scotland. The liver has an enormous capacity for wound repair and regeneration after injury, but early diagnosis is essential. Despite the high number of deaths each year from liver disease, hepatologists still lack relevant diagnostic biomarkers to detect early liver disease.

The aim of this project is to identify a suitable biomarker to detect early liver disease. Insulin-like growth factor binding protein-5 (IGFBP-5) is one such potential biomarker. IGFBP-5 has already been shown to be upregulated in skin fibrosis (Yasuoka et al., 2006a), fibrosis of the lung (Yasouka et al., 2006b) and has been identified as a genetic marker for intrahepatic cholangiocarcinoma (Blechacz and Gores, 2008). Its role in liver damage is being investigated using an in vitro model of chronic low level toxic insult in primary cultures of rat hepatocytes (male Sprague–Dawley rats, 180–220 g).

The effects of low concentrations of two oxidative toxins (Menadione (0.1, 1, 5 and 10 μM) and hydrogen peroxide (0.01, 0.05,

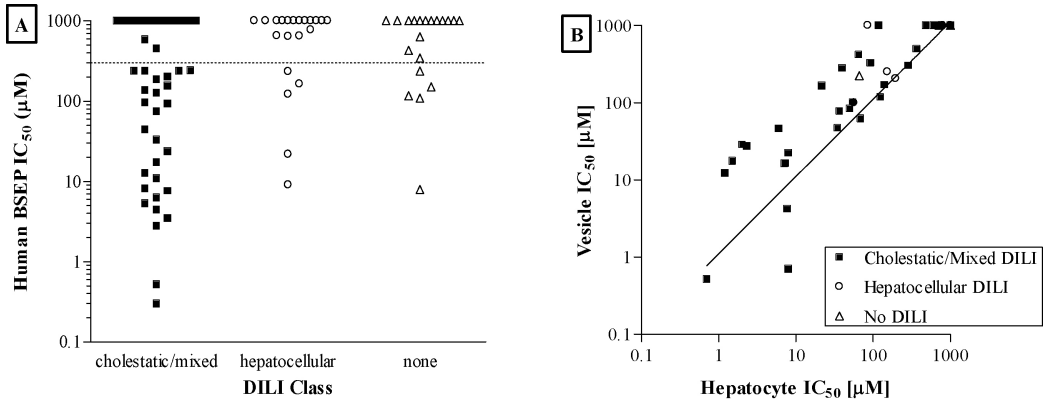

Fig. 1. Inhibition of rat Bsep activity by DILI compounds in vesicles (A) and correlation of IC_{50} values between vesicles and hepatocytes (B).

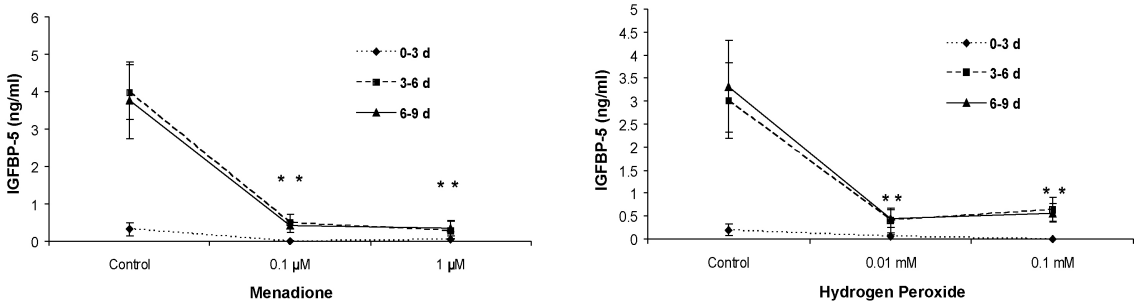

Fig. 1. IGFBP-5 release from primary rat hepatocytes exposed to Menadione and hydrogen peroxide was measured using media samples collected over 0–3 days, 3–6 days and 6–9 days (results are mean ± SEM, $n = 3$, $^{*}P < 0.05$, by one-way ANOVA followed by Dunnett's multiple comparison test).

0.1 and 0.5 mM)) were assessed over a period of 9 days in culture. Fresh toxin was added daily and the Williams' E medium changed every three days. Toxicity via oxidative stress was assessed by measuring reduced glutathione (GSH) content and WST-1 assay (Roche Applied Science, cat. no. 11 644 807 001). The cell number was assessed using crystal violet (CV) staining and IGFBP-5 secretion into the medium was measured using a sandwich ELISA assay.

Oxidative stress was observed and both toxins caused a significant decrease in GSH content. After 4 days exposure to 5 μM Menadione, GSH content had declined to 2.97 ± 0.20 nmol/well compared with controls (3.73 ± 0.03 nmol/well). The corresponding value after 0.01 mM H_2O_2 exposure was 3.40 ± 0.21 nmol/well in comparison with controls (4.22 ± 0.18 nmol/well) ($P < 0.05$). Cell death was not observed until 9 days exposure to the toxins when CV staining was significantly decreased ($P < 0.05$). IGFBP-5 secretion into the medium was lower in samples from cells following exposure to toxins than in the control samples taken at the same time points (Fig. 1). It should be noted that IGFBP-5 secretion increased in the control samples between 3 and 9 days in culture ($P < 0.05$). These results suggest that IGFBP-5 secretion may be associated with de-differentiation, and have a role in the decline of cellular function in hepatocytes during culture, but does not appear to be involved in toxicity by oxidative stress as induced by Menadione and hydrogen peroxide.

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Protein binding and pharmacokinetics of reactive acyl glucuronide drug metabolites

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Tolmetin and diclofenac are members of the non-steroidal anti-inflammatory class of drugs (NSAIDs). Many NSAIDs have been associated with idiosyncratic toxicity, manifesting in hepatotoxicity or hypersensitivity reactions. These adverse reactions have been suggested to be a result of bioactivation to protein reactive metabolites. It is hypothesised that acyl glucuronide (AG) metabolites are sufficiently protein reactive to elicit an idiosyncratic reaction through the initiation of an immune response (Bailey and Dickenson, 2003). We have investigated this possibility through the incubation of the synthetic AG metabolites of diclofenac and tolmetin with human, rat and mouse serum albumin. We have investigated the metabolism of two NSAIDs into their AG metabolites, their AG-mediated protein binding, and are currently investigating rates of AG clearance in the rat.

Tolmetin acyl glucuronide (T-AG) and diclofenac acyl glucuronide (D-AG) were incubated with human serum albumin (HSA) at increasing concentrations for 16 h at 37 °C, with AG covalent adduct formation assessed using LC–MS/MS technology (AB Sciex QTRAP 5500.)

Male Wistar rats were anaesthetized using 14% urethane, with the bile duct and carotid artery cannulated. Tolmetin was then administered orally at a dose of 10 mg/kg, with bile and blood samples collected over 4 h for pharmacokinetic analysis ($n = 3$). Following anesthesia, the femoral vein of male Wistar rats were cannulated with D-AG administered through this route at a dose of 60 mg/kg. Blood samples were collected, and albumin was isolated using preparative HPLC. Adducts were searched for using an AB Sciex QTRAP 5500 LC–MS/MS (Table 1).

Following *in vitro* incubation of T-AG with rat (RSA) and mouse (MSA) serum albumin, following the same procedure as for HSA, fewer amino acids were found to be modified at higher concentrations and adducts were not found at lower concentrations of 1:1 molar ratios, unlike following incubation with HSA. No albumin adducts were detected following i.v. administration of D-AG at