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Both cholestatic and steatotic drugs trigger extensive alterations in the mRNA level of biliary transporters in rat hepatocytes: Application to develop new predictive biomarkers for early drug development

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HIGHLIGHTS

- The mechanisms responsible for drug-induced cholestasis are not fully understood.
- Cholestatic drugs disturb the mRNA level of most hepatocyte biliary transporters.
- The majority of steatotic drugs also affect the expression of these genes.
- OATP1A1 mRNA could be a biomarker for predicting new cholestatic/steatotic drugs.
- The mechanisms for iatrogenic cholestasis and steatosis may have common features.

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ABSTRACT

Disruption of the vectorial bile acid transport in the liver is a key feature of cholestatic drugs, although many causal and mechanistic aspects are still unknown. The aim of the present study was to explore if cholestatic drugs can repress or induce the expression of hepatic transporters. To this end, sandwich-cultured rat hepatocytes were treated with cholestatic and non-cholestatic (steatotic, non-hepatotoxic, etc.) drugs and the mRNA expression of 10 uptake and efflux biliary transporters was measured. Results evidenced that all cholestatic drugs cause extensive alterations in the mRNA expression of most biliary transporters. Surprisingly, nearly all steatotic drugs also affected the expression of these genes. The most frequent alterations triggered by both types of drugs were the repression of OATP1A1, NTCP and BSEP, and the induction of MRP2/3/4, MDR2 and ABCG5/8. The majority of these alterations were also observed in vivo, in the livers of treated rats. The common signature of cholestatic and steatotic drugs was the repression of OATP1A1. Indeed, ROC curve analysis indicated that OATP1A1 mRNA is a very sensitive marker to identify drugs with cholestatic or steatotic potential, with a maximal sensitivity and specificity of 0.917 and 0.941, respectively. We conclude that alteration of expression of hepatobiliary transporters is a hallmark of both cholestatic and steatotic drugs, lending support to a connection between these two mechanisms of hepatotoxicity. Moreover, OATP1A1 mRNA is proposed as a very simple and useful screening biomarker for the prediction of new cholestatic or steatotic drugs in early drug development.

Abbreviations: ANIT, α-Naphthyl isothiocyanate; AMIK, amikacin; AMIO, amiodarone hydrochloride; AMIT, amitriptyline; AUC, area under the curve; AZAT, azathioprine; BA, bile acid; BOSE, Bosentan; C, cholestatic drug; CHLO, chlorpromazine; CITR, citrate; CYCA, cyclosporine A; DILI, drug-induced liver injury; DOXY, doxycycline; DMSO, dimethyl sulfoxide; ETHY, ethynilestradiol; FENO, fenofibrate; GLYB, glyburide; KETO, ketotifen; MAPR, maprotiline; METF, metformin; NAFLD, non-alcoholic fatty liver disease; NCNS, non-cholestatic/non-steatotic compound; NPV, negative predictive values; PERH, perhexiline; NR, nuclear receptors; PPV, positive predictive values; SCH, sandwich-cultured hepatocytes; ROC, receiver operating characteristic; SIMV, simvastatin; S, steatotic drugs; TAMO, tamoxifen; TICL, ticlopidine; TROG, troglitazone; TETR, tetracycline hydrochloride; TIAN, tianeptine; VALP, sodium valproate; ZIDO, zidovudine.

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1. Introduction

The term drug-induced liver injury (DILI) comprises different manifestations of liver toxicity following drug exposure and is one of the leading causes of acute liver failure, and the most common reason for market withdrawals and safety warnings of approved drugs (Bjornsson et al., 2013; Reuben et al., 2010). Cholestatic, hepatocellular (inflammatory) and mixed injuries are the three most common and severe manifestations of DILI (Bjornsson et al., 2013; Padda et al., 2011). Cholestasis is defined as impaired bile formation or excretion by hepatocytes and is characterized by accumulation of bile acids (BAs) in systemic blood and within hepatocytes. When the concentration of BAs exceeds the binding capacity of the cytosolic binding proteins, BAs induce hepatocyte apoptosis and necrosis, by damage to mitochondria (Palmeira and Rolo, 2004). Early detection of drug-induced cholestasis remains a challenge during drug development. Currently existing in vitro systems show poor predictivity towards the clinical situation. Therefore, the pharmaceutical industry is currently focusing on developing reliable and accurate in vitro assays to deselect compounds with cholestatic potential early in drug discovery.

Bile is a complex mixture that includes BAs, phospholipids, cholesterol, conjugated bilirubin and drugs. Hepatocytes are polarized cells that have specialized transporters in the sinusoidal/basolateral and canalicular/apical membranes to maintain the enterohepatic circulation of BA and the canalicular efflux of the different bile components. The sodium taurocholate cotransporting polypeptide (NTCP/SLC10A1) accounts for the major uptake of conjugated BAs (Hagenbuch and Dawson, 2004), although other transporters are also involved (e.g. OATPs/SLCOs). The apically localized bile salt export pump (BSEP/ABCB11) is the primary transporter involved in the biliary efflux of conjugated BAs across the canalicular membrane (Byrne et al., 2002). In addition to BSEP, canalicular (MRP2/ABCC2) and basolateral (MRP3/4/ABCC3/4) multidrug resistance-associated proteins (MRPs) are also involved in conjugated BA export (Dawson et al., 2009). The other essential transporters involved in bile secretion from hepatocytes into the canaliculi are the multidrug resistance protein MDR2/3 (ABCB4) for phospholipids (Morita and Terada, 2014), the ABC subfamily G members, ABCG5 and ABCG8, for cholesterol (Klaassen and Aleksunes, 2010), and MRP2 and BCRP1 (ABCG2) for bilirubin glucuronides (Kamisako et al., 2000).

In addition to their involvement in the transport of BAs and other endogenous substrates, these basolateral and canalicular transporter systems are also involved in the transport of drugs and xenobiotics. NTCP and BSEP are predominantly BA transporters, but they can also transport, for instance, the statins rosuvastatin and pravastatin, respectively. On the contrary, OATPs are drug rather than BA transporters mediating the uptake of a multitude of xenobiotics (Stieger and Geier, 2011). This can potentiate the intracellular accumulation of BAs and result in the development of cholestatic liver damage (Fattinger et al., 2001; Morgan et al., 2010). Indeed, hepatic transport proteins are important targets of toxic interactions, and inhibition of basolateral transport and/or canalicular excretion of BAs by drugs or metabolites is a well-recognized cause of cholestasis (Dawson et al., 2012; Fattinger et al., 2001; Kock et al., 2014; Morgan et al., 2010).

More specifically, direct inhibition of BSEP has been proposed as a major mechanism for drug-induced cholestasis, and a number of assays either in sandwich-cultured hepatocytes (SCH) or BSEP-expressing membrane vesicles have been proposed to evaluate the potential of drugs to disrupt BSEP function (Dawson et al., 2012; Kostrubsky et al., 2003; McRae et al., 2006; Morgan et al., 2013). However, BSEP inhibition has been shown insufficient to predict the cholestatic potential of all drugs, and other mechanisms (e.g., inhibition of other BA transporters and enzymes, defective localization or alteration of gene expression) may also take place (Kock et al., 2014; Morgan et al., 2013; Rodrigues et al., 2014).

Collagen SCH maintain the expression and function of key uptake and efflux transporters relative to in vivo, and it is recognized as the most relevant system to evaluate and predict the potential of a compound to cause transporter-based liver toxicity (Godoy et al., 2013). The aim of the present study was to investigate if cholestatic drugs can repress or induce the expression of hepatic transporters using rat SCH as experimental model. To this end, a transcriptome analysis of biliary uptake (NTCP, OATP1A1) and efflux (BSEP, MRP2/3/4, MDR2, BCRP1, ABCG5/8) transporters upon drug exposure was performed. The final goal was to explore new mechanisms for drug-induced cholestasis and novel predictive biomarkers for this hepatotoxic outcome that could be useful as a screening tool in early drug development.

2. Material and methods

2.1. Chemicals

Test compounds and other chemicals used were purchased from the following sources: amikacin, amiodarone hydrochloride, amitriptyline, α-naphthyl isothiocyanate (ANIT), azathioprine, chlorpromazine, cyclosporine A, DMSO, doxycycline hydrochloride, ethynylestradiol, fenofibrate, glyburide, ketotifen, maprotiline hydrochloride, metformin, perhexiline, simvastatin, tamoxifen, tianeptine sodium salt, ticlopidine, tetracycline hydrochloride, troglitazone and sodium valproate from Sigma (Madrid, Spain); sodium citrate from Merck (Madrid, Spain); and zidovudine from Glaxo Smith Kline (Madrid, Spain). Bosentan was chemically extracted from Tacleer® tablets (Actelion Pharmaceuticals Ltd).

2.2. Hepatocyte isolation and culture

Hepatocytes were isolated from male Sprague & Dawley rats (180–250 g; Charles River, France), by two-step in situ perfusion of the liver with collagenase, as described previously in detail (Gomez-Lechon et al., 2006). Cellular viability was estimated by the trypan blue dye exclusion test. Hepatocytes were then plated on 24-well Biocoat™ collagen I plates at a density of 150000 cells/well in 1.5 ml Ham's F-12/Williams (1:1) medium (Gibco BRL, Paisley, Scotland), supplemented as described (Gomez-Lechon et al., 2006). Cells were incubated at 37 °C, 5% CO_2 in a humidified incubator and the medium was change 1 h later to remove unattached hepatocytes. One day after plating the medium was removed and the cells overlaid with type I collagen solution (25 μl/well). Collagen solution was prepared immediately before use by mixing 9 vol. of a 1.87 mg/ml type I collagen (Roche, Germany) in 0.1% acetic acid and 1 vol. of 10 x DMEM medium (Koebe et al., 1994). Serum-free hormone-supplemented plating medium (10^{-9} M dexamethasone and insulin) was pipetted on the cells and changed every 24 h thereafter (Gomez-Lechon et al., 2006).

2.3. Selection of compounds and treatments

Twenty-five compounds were included in the study (Table 1), which were chosen according to previous information on both their hepatotoxic potential and their reported cholestatic (n = 10) or steatotic (n = 8) effect (Gomez-Lechon et al., 2010). Additionally, sodium citrate, amikacin, amitriptyline, ketotifen, maprotiline, metformin and simvastatin were used as negative (non cholestatic/non steatotic) controls (Table 1). The selected concentrations did not cause apparent cytotoxicity and ranged up to 100-fold the therapeutic peak plasmatic concentration (C_{max}), when available, which is a cutoff currently used for safety assessment in