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LETTER TO THE EDITOR, NEWS AND VIEWS

In vitro prediction of drug-induced cholestatic liver injury: a challenge for the toxicologist

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Abbreviations

AOP(s)	Adverse outcome pathway(s)
DILI	Drug-induced liver injury
KE(s)	Key event(s)
KER(s)	Key event relationship(s)
MIE(s)	Molecular initiating event(s)

Cholestasis is derived from the Greek words *chole* meaning bile and *stasis* indicating halting (Noor 2015), and denotes any situation of impaired bile secretion with concomitant accumulation of bile acids in the liver or in the systemic circulation. A variety of factors may evoke cholestasis, including genetic disorders, metabolic pathologies, infectious diseases, immunogenic stimuli and drugs (Anthérieu et al. 2013; Gossard and Talwalkar 2014; Nguyen et al. 2014; Noor 2015). Drugs can induce either acute or chronic cholestasis, whereby symptoms resolve upon drug withdrawal or persist for periods over 6 months despite drug retraction, respectively. Acute drug-induced cholestasis occurs most frequently and manifests with or without hepatocellular and inflammatory injury, and is associated with vague symptoms, including nausea, malaise, anorexia and fatigue. Chronic drug-induced cholestasis occurs as a result of injury to bile ducts or ductules with clinical features such as pruritus, jaundice, melanoderma and xanthoma formation (Bhamidimarri and Schiff 2013; Gossard and Talwalkar 2014; Yang et al. 2013).

Drug-induced cholestasis constitutes a subgroup of drug-induced liver injury (DILI). DILI is a major reason of drug failure during premarketing and postmarketing phases, accounting for up to 29% of all drug withdrawals (Lee 2013; Van den Hof et al. 2015). In addition to its pharmaceutical relevance, DILI is also of high clinical concern. Indeed, DILI is frequently misdiagnosed, yet it has been estimated to develop in 1 in 100 patients during hospitalization (Meier et al. 2005). Furthermore, DILI is responsible for more than 50% of all cases of acute liver failure (Goldberg et al. 2015). As such, 20–40% and 12–20% of DILI patients presents a cholestatic and mixed hepatocellular/cholestatic injury pattern, respectively (Bhamidimarri and Schiff 2013; Sharanek et al. 2016). Cholestatic DILI is seen most frequently among men over 60 years old (Meier et al. 2005). The overall mortality rate of DILI attributed to cholestasis is 2.5–7.8% (Bhamidimarri and Schiff 2013; Björnsson and Olsson 2005; Noor 2015; Sharanek et al. 2016; Wolters et al. 2016). Although more than 1 drug can be involved in DILI, single prescription medication underlies 73% of all drug-induced cholestasis cases. More than 1000 drugs have been associated with cholestatic liver injury, including anti-infectious drugs, anti-diabetics, anti-inflammatory drugs, psychotropic drugs, cardiovascular drugs and steroids (Bhamidimarri and Schiff 2013; Parmentier et al. 2017).

Preclinical animal models only allow to predict 50–60% of human DILI cases because of interspecies differences. Likewise, current human-based hepatic in vitro models merely pick up half of clinical DILI events (Laverty et al. 2010; Xu et al. 2004). The latter obviously can be attributed to overall in vitro–in vivo differences, but may also be due to gaps in the mechanistic understanding of DILI, *in casu* cholestasis. A pragmatic tool to rationally and visually capture existing knowledge regarding the mechanistic basis of toxicity includes the so-called adverse outcome pathway (AOP), which starts from a molecular initiating event (MIE) (i.e. a trigger of toxicity) and that relies on a series of key events (KEs), linked by key event relationships (KERs), ultimately resulting in a specific toxicological effect (Ankley et al. 2010; Gijbels and Vinken 2017; Leist et al. 2017;

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Vinken et al. 2017; Vinken 2013, 2015, 2016). A number of AOPs related to hepatotoxicity, including liver steatosis, liver fibrosis and cholestasis, have been proposed (Gijbels and Vinken 2017). Most currently available AOPs consider only 1 MIE. Clearly, this is not a full reflection of the actual in vivo situation, as toxicological effects are not as straightforward as depicted in AOPs. An improvement came with the introduction of AOP networks, which combine different AOPs that share at least 1 KE (Villeneuve et al. 2014). Such AOP networks have been described for liver steatosis and seem to provide an in vivo-complying mechanistic scenario (Mellor et al. 2016). Other types of hepatotoxicity, however, in particular cholestasis, are more complex. Indeed, bile accumulation triggers 2 types of cellular responses, namely an adverse response and an adaptive response, which occur in parallel and that each are typified by a number of KEs (Vinken et al. 2013). Thus, the adverse response is accompanied by the onset of processes such as oxidative stress (Allen et al. 2010; Cai et al. 2017; Kim et al. 2006; Tan et al. 2010; Woolbright and Jaeschke 2012), inflammation (Gong et al. 2016; Li et al. 2017) and different cell death modes (Gujral et al. 2004; Mitchell et al. 2011; Woolbright et al. 2013, 2015). On the other hand, the adaptive response, aimed at decreasing the uptake and increasing the export of bile acids into and from hepatocytes, respectively, depends on the activation of several nuclear receptors, including the farnesoid X receptor, the pregnane X receptor and the constitutive androstane receptor (Boyer 2009; Cuperus et al. 2014; Wagner et al. 2009; Zollner and Trauner 2006, 2008). As a result, mechanistic modelling of cholestasis in AOP networks is challenging, as this should take into account the entangled pathways that drive these cellular responses as taking place in vivo. Furthermore, a number of potentially new cholestatic MIEs, like altered bile canaliculi dynamics (Burban et al. 2017; Burbank et al. 2016; Sharanek et al. 2016), as well as KEs, such as necroptosis and autophagy (Afonso et al. 2016; Gao et al. 2014; Lin et al. 2012; Manley et al. 2014; Sasaki et al. 2015), have been identified in the last few years. This adds to the mechanistic complexity of cholestasis and more mechanisms may emerge in the upcoming years given the worldwide increasing research efforts in this area.

AOPs and their networks can serve as the basis for setting up batteries of in vitro tests, each that detects 1 or more KEs, and which collectively may enable accurate prediction of toxicity inflicted by chemicals. A prerequisite in this respect is the use of in vitro models that appropriate reproduce in vivo cholestatic liver injury. A number of state-of-the-art in vitro models to study hepatotoxicity induced by chemicals belonging to 2 classes are presently available, namely liver-derived in vitro models and stem cell-derived in vitro models. Stem cell-derived in vitro models have shown their value for studying different types of liver toxicity, including liver steatosis (Pradip et al. 2015; Rodrigues et al. 2014). However, although some studies demonstrated their promise (Ghodsizadeh et al. 2010; Imagawa et al. 2017), hepatocyte-like cells derived from induced pluripotent stem cells have been reported not to be the most appropriate in vitro systems for the detection of cholestatic chemicals (Bell et al. 2017), which is due in part to their inability to properly trigger the adaptive response, being critical for the actual manifestation of cholestatic liver toxicity. This is unlike most liver-derived in vitro models (Godoy et al. 2013), which are more suitable tools for the screening of cholestatic compounds. These liver-derived in vitro models include cultures of human hepatoma HepaRG cells (Woolbright et al. 2016), freshly isolated human liver slices (Vatakuti et al. 2017), primary human hepatocytes "sandwiched" (i.e. cultured) between 2 layers of extracellular matrix components (Chatterjee et al. 2014; Oorts et al. 2016) and spheroid cultures of primary human hepatocytes (Bell et al. 2016; Hendriks et al. 2016). Because of their longevity, the presence of an in vivo-like tridimensional cellular configuration and cellular interactions as well as of bile ductules (Fraczek et al. 2013), sandwich and spheroid cultures of primary human hepatocytes are currently considered as the best performing in vitro systems to detect cholestatic compounds. In fact, it has been shown that the sensitivity for cholestatic effects in spheroid cultures of primary human hepatocytes increases with exposure time (Hendriks et al. 2016), which may be associated with the occurrence of the adaptive response.

Future strategies to improve in vitro predictivity of in vivo drug-induced cholestatic liver injury should in first instance focus on the full elucidation of the underlying mechanisms of cholestasis in an AOP network framework. This will yield series of KE-specific biomarkers, which can be picked up by "omics" technologies and that altogether can form a mechanistic signature of drug-induced cholestatic liver injury. The resulting AOP network on cholestasis should be ideally embedded in structures that also consider other aspects of toxicity, in particular kinetics and exposure parameters. Simultaneously, current in vitro models must be further improved for application in the detection of cholestatic potential of chemicals. This particularly holds for stem cell-derived in vitro models. Furthermore, in vitro testing approaches should be complemented with emerging in silico methods that computationally predict cholestatic potential based on chemical structures and/or physico-chemical profiles. It can be anticipated that full integration of new knowledge and methodologies in the upcoming years will enable early and accurate detection of the cholestatic potential of drugs and chemicals in general. This will not only increase human chemical safety as such, but will equally reduce and even fully replace the use of animals for toxicity testing.

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