Highlight report: prediction of drug induced liver injury (DILI) with human hepatocytes in vitro

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Recently, William Proctor and colleagues have published a comprehensive study about the use human hepatocyte spheroids to predict DILI in patients (Proctor et al. 2017). This is a highly relevant topic, since DILI remains a major challenge in toxicology (Godoy et al. 2013; Björnsson 2015; Dragovic et al. 2016; Ghallab 2014). One of the conclusions of the authors is that three-dimensional human hepatocyte spheroid cultures allowed a higher sensitivity compared to human hepatocytes that were simply cultivated on collagen coated dishes (Proctor et al. 2017). However, this is not surprising, since similar observations have been reported in the past (Hewitt et al. 2007; Hengstler et al. 2000; Deharde et al. 2016; Luckert et al. 2017; Rebelo et al. 2015). However, the set of data presented by the authors is of high relevance. They report cytotoxicity of human hepatocyte spheroids concentration-dependently incubated with 110 drugs for 14 days. These drugs have been categorized into: (1) severe clinical DILI concern, (2) high clinical DILI concern, (3) low clinical DILI concern, (4) enzyme elevations in the clinic, (5) no DILI concern. Moreover, the maximal plasma concentrations ($C_{\max}$) of these drugs have been reported. Using the DILI severity category and exposure-corrected cytotoxicity, the authors report that hepatocyte spheroids can be used for classifying hepatotoxicants from different pharmacological classes (Proctor et al. 2017).

Particularly relevant conclusions can be drawn from the electronic supplemental material. In Proctor et al. 2017, the Supplementary table S-3 cytotoxicity data (IC_{50}) and the human plasma peak concentrations ($C_{\max}$) have been summarized (Table S-3, S3 hLiMT data). For some compounds the in vitro IC_{50} and plasma peak concentrations are in the same order of magnitude. For example the category 1

(severe DILI concern) compound ketoconazole has an IC_{50} of 4.6 μM and a $C_{\max}$ of 11.3 μM . This results in a ratio ($IC_{50}/C_{\max}$) of 0.4, also named ‘margin of safety’ (MOS). Therefore, the in vitro system with hepatocyte spheroids predicts the in vivo situation for ketoconazole quite well. However, the situation is difficult for other compounds with severe DILI concern (category 1): ximelagatran shows an in vitro IC_{50} of 150 μM , but the human plasma peak concentration ($C_{\max}$) is only 0.45 μM . Therefore, the risk of human DILI has been massively underestimated by the in vitro system. Similar problems exist for the category 1 compounds dantrolene, methotrexate, stavudine and trovafloxacin (Proctor et al. 2017; Table S-3, S3h LiMT data). It is interesting to note that cytotoxicity allows a good prediction of human blood concentrations associated with an increased risk of DILI for some compounds, while it leads to a massive underestimation of other severe DILI compounds.

In recent years, much effort has been invested into the development of hepatocyte in vitro systems (Ehrhardt and Schmicke 2016; Ramboer et al. 2015; Verhulst et al. 2015; Hammad and Ahmed 2014; Hammad et al. 2017; Godoy et al. 2016, 2015; Grinberg et al. 2014). Besides cytotoxicity further readouts, such as gene expression or functional endpoints have been recommended (Grinberg et al. 2014; Reif et al. 2015; Rashidi et al. 2016; Arbo et al. 2016). However, a comprehensive study including a sufficiently large number of compounds for validation of these endpoints is not yet available. The merit of the present study (Proctor et al. 2017) is that it delivers a comprehensive cytotoxicity database, which clearly demonstrates that cytotoxicity alone is not sufficient to predict human DILI. In conclusion, the hunt for an accurate in vitro system quantitatively predicting human hepatotoxicity has only just begun.

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