GUEST EDITORIAL

Future perspectives of DILI prediction in vitro

Ahmed Ghallab[1] · Abdel-latif Seddek[1]

The publication of Proctor et al. (2017) about prediction of drug-induced liver injury by human hepatocyte spheroid cultures belongs to the most cited studies published in the Archives of Toxicology in 2017. The authors tested cytotoxicity of 110 drugs (hepatotoxic and non-hepatotoxic) and compared EC_{50} values to the C_{max} of the corresponding compounds in human plasma. The margin of safety concept (MOS) defines a compound as positive (toxic) in vitro if $EC_{50} < C_{max} \times T$, whereby T represents an arbitrarily set threshold that typically ranges between 10 and 100. With a threshold of 10, the authors obtained a sensitivity of 36.2% and a specificity of 97.6% (Proctor et al. 2017). Using a threshold of 100, sensitivity and specificity were 59.4 and 80.5%, respectively. Although the study represents an important milestone, these numbers also illustrate a major remaining challenge; even with the very high threshold of 100 a relatively high fraction of in vivo hepatotoxic compounds is predicted as negative by the in vitro test. Several other studies used similar approaches and reported similar limitations (Xu et al. 2008; Khetani et al. 2013; Albrecht et al. 2019; Gu et al. 2018; Frey et al. 2014; Hewitt et al. 2007).

Mechanisms leading to hepatotoxicity are complex (Zárybnický et al. 2018; Hammad et al. 2017; Hoehme et al. 2010); often hepatocyte death is a primary key event (Ghallab et al. 2019; Leist et al. 2017; Sachinidis et al. 2019). However, also toxic effects to cholangiocytes endothelial cells or immune cells may represent initiating events (Godoy et al. 2013; Connolly et al. 2011; Stout-Delgado et al. 2007; Stachlewitz et al. 1999). It has become clear that cytotoxicity represents a highly relevant readout of in vitro tests with cultivated hepatocytes that is sufficient to identify at least 50% of all hepatotoxic compounds. However, for identification of a relatively large fraction of hepatotoxic compounds, cytotoxicity alone is inadequate. In future, additional readouts should be determined in test batteries to study whether they improve sensitivity without reducing specificity. Possible candidates of additional readouts are inhibition of bile salt export (Godoy et al. 2013; Jansen et al. 2017), altered gene expression (Grinberg et al. 2014; Godoy et al. 2016), or secretion of cytokines that may activate immune cells (Wewering et al. 2017). Systematic studies analyzing these additional readouts with respect to sensitivity and specificity are not yet available. However, the already existing cytotoxicity-based studies offer a good basis, since they have identified a relatively large set of compounds, whose hepatotoxicity cannot be sufficiently evaluated by cytotoxicity alone.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Albrecht W, Kappenberg F, Brecklinghaus T et al (2019) Prediction of human drug-induced liver injury (DILI) in relation to oral doses and blood concentrations. Arch Toxicol 93:1609–1637. https://doi.org/10.1007/s00204-019-02492-9

Connolly MK, Ayo D, Malhotra A et al (2011) Dendritic cell depletion exacerbates acetaminophen hepatotoxicity. Hepatology 54(3):959–968. https://doi.org/10.1002/hep.24429

Frey O, Misun PM, Fluri DA, Hengstler JG, Hierlemann A (2014) Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis. Nat Commun 5:4250. https://doi.org/10.1038/ncomms5250

Ghallab A, Hofmann U, Sezgin S et al (2019) Bile microinfarcts in cholestasis are initiated by rupture of the apical hepatocyte membrane and cause shunting of bile to sinusoidal blood. Hepatology 69(2):666–683

Godoy P, Hewitt NJ, Albrecht U et al (2013) Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. Arch Toxicol 87(8):1315–1530

✉ Ahmed Ghallab
Ghallab@vet.svu.edu.eg

[1] Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

Godoy P, Widera A, Schmidt-Heck W et al (2016) Gene network activity in cultivated primary hepatocytes is highly similar to diseased mammalian liver tissue. Arch Toxicol 90(10):2513–2529

Grinberg M, Stöber RM, Edlund K et al (2014) Toxicogenomics directory of chemically exposed human hepatocytes. Arch Toxicol 88(12):2261–2287

Gu X, Albrecht W, Edlund K et al (2018) Relevance of the incubation period in cytotoxicity testing with primary human hepatocytes. Arch Toxicol 92(12):3505–3515. https://doi.org/10.1007/s00204-018-2302-0

Hammad S, Braeuning A, Meyer C, Mohamed FEZA, Hengstler JG, Dooley S (2017) A frequent misinterpretation in current research on liver fibrosis: the vessel in the center of CCl₄-induced pseudolobules is a portal vein. Arch Toxicol 91(11):3689–3692

Hewitt NJ, Lechón MJ, Houston JB et al (2007) Primary hepatocytes: current understanding of the regulation of metabolic enzymes and transporter proteins, and pharmaceutical practice for the use of hepatocytes in metabolism, enzyme induction, transporter, clearance, and hepatotoxicity studies. Drug Metab Rev 39(1):159–234 **(HMPC/EMA138386/2005 Rev. 1 C)**

Hoehme S, Brulport M, Bauer A et al (2010) Prediction and validation of cell alignment along microvessels as order principle to restore tissue architecture in liver regeneration. Proc Natl Acad Sci USA 107(23):10371–10376

Jansen PL, Ghallab A, Vartak N, Reif R, Schaap FG, Hampe J, Hengstler JG (2017) The ascending pathophysiology of cholestatic liver disease. Hepatology 65(2):722–738

Khetani SR, Kanchagar C, Ukairo O et al (2013) Use of micropatterned cocultures to detect compounds that cause drug-induced liver injury in humans. Toxicol Sci 132(1):107–117

Leist M, Ghallab A, Graepel R et al (2017) Adverse outcome pathways: opportunities, limitations and open questions. Arch Toxicol 91(11):3477–3505. https://doi.org/10.1007/s00204-017-2045-3 **(Review)**

Proctor WR, Foster AJ, Vogt J et al (2017) Utility of spherical human liver microtissues for prediction of clinical drug-induced liver injury. Arch Toxicol 91(8):2849–2863. https://doi.org/10.1007/s00204-017-2002-1

Sachinidis A, Albrecht W, Nell P, Cherianidou A, Hewitt NJ, Edlund K, Hengstler JG (2019) Road map for development of stem cell-based alternative test methods. Trends Mol Med 25(6):470–481. https://doi.org/10.1016/j.molmed.2019.04.003 **(Review)**

Stachlewitz RF, Seabra V, Bradford B, Bradham CA, Rusyn I, Germolec D, Thurman RG (1999) Glycine and uridine prevent D-galactosamine hepatotoxicity in the rat: role of Kupffer cells. Hepatology 29(3):737–745

Stout-Delgado HW, Getachew Y, Rogers TE, Miller BC, Thiele DL (2007) The role of serpinb9/serine protease inhibitor 6 in preventing granzyme B-dependent hepatotoxicity. Hepatology 46(5):1530–1540

Wewering F, Jouy F, Wissenbach DK et al (2017) Characterization of chemical-induced sterile inflammation in vitro: application of the model compound ketoconazole in a human hepatic co-culture system. Arch Toxicol 91(2):799–810. https://doi.org/10.1007/s00204-016-1686-y

Xu JJ, Henstock PV, Dunn MC, Smith AR, Chabot JR, de Graaf D (2008) Cellular imaging predictions of clinical drug-induced liver injury. Toxicol Sci 105(1):97–105

Zárybnický T, Boušová I, Ambrož M, Skálová L (2018) Hepatotoxicity of monoterpenes and sesquiterpenes. Arch Toxicol 92(1):1–13. https://doi.org/10.1007/s00204-017-2062-2

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.