

## Guest editorial:

# HIGHLIGHT REPORT: METABOLOMICS IN HEPATOTOXICITY TESTING

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Hepatotoxicity is one of the most frequent forms of systemic toxicity of drugs and a leading cause for drug withdrawal from the market. To improve the possibilities to predict hepatotoxicity, Tzutzuy Ramirez and colleagues from BASF in Ludwigshafen recently tested a metabolomics based *in vitro* system (Ramirez et al., 2017). They exposed HepG2 cells to 35 test compounds and applied LC-MS/MS as well as GC-MS to quantify 89 metabolites in the culture medium supernatant and 194 intracellular metabolites. A main focus was to determine quality criteria, such as reproducibility and concentration dependency of the test system. The relative standard deviations of technical replicates were in the range of 5-10 %, while controls from different days were between 10 and 15 % (Ramirez et al., 2017). This is an excellent reproducibility for an *in vitro* system. Moreover, convincing concentration-response relationships were obtained and metabolite patterns could be associated with specific mechanisms of toxicity, such as peroxisome proliferation and liver enzyme induction or inhibition (Ramirez et al., 2017). Therefore, the HepG2 metabolomics technique represents a promising new candidate in the field of *in vitro* hepatotoxicity prediction. Limitations are that the HepG2 cells show major metabolic differences compared

to human hepatocytes. Moreover, the present study did not yet include a systematic comparison of negative and positive controls at *in vivo* relevant concentrations, which remains a challenge for the future.

Currently, mechanisms of hepatotoxicity represent a major focus in toxicological research (Kyriakides et al., 2016; Ghallab, 2015c; Ramachandran et al., 2015; Chen et al., 2015; Campos et al., 2014; Hammad et al., 2014; Hassan, 2016; Stöber, 2015). Despite progress in the field of stem cell research (Gómez-Lechón and Tolosa, 2016; Godoy et al., 2016; Cameron et al., 2015) and studies with cell lines (Tolosa et al., 2015; Hewitt et al., 2007; Godoy et al., 2013), primary hepatocytes still remain a gold standard (Reif et al., 2015; Grinberg et al., 2014; Stöber, 2015; Ghallab, 2015a; Arbo et al., 2016). Moreover PBPK modeling (Ghallab, 2015b; Reif et al., 2017; Thiel et al., 2015) and spatio-temporal models (Ghallab et al., 2016; Vartak et al., 2016; Jansen et al., 2017; Friebel et al., 2015) have supported our understanding of the mechanisms of liver toxicity. The next year will show whether the novel HepG2 metabolomics assay can be integrated into useful test batteries for a better prediction of human hepatotoxicity.

## REFERENCES

- Arbo MD, Melega S, Stöber R, Schug M, Rempel E, Rahnenführer J, et al. Hepatotoxicity of designer drugs: up-regulation of key enzymes of cholesterol and lipid biosynthesis. *Arch Toxicol.* 2016;90:3045-60.
- Cameron K, Tan R, Schmidt-Heck W, Campos G, Lyall MJ, Wang Y, et al. Recombinant laminins drive the differentiation and self-organization of derived hepatocytes. *Stem Cell Rep.* 2015;5:1250-62.
- Campos G, Schmidt-Heck W, Ghallab A, Rochlitz K, Pütter L, Medinas DB, et al. The transcription factor CHOP, a central component of the transcriptional regulatory network induced upon CCl<sub>4</sub> intoxication in mouse liver, is not a critical mediator of hepatotoxicity. *Arch Toxicol.* 2014;88:1267-80.
- Chen R, Wang J, Zhang Y, Tang S, Zhan S. Key factors of susceptibility to anti-tuberculosis drug-induced hepatotoxicity. *Arch Toxicol.* 2015;89:883-97.
- Friebel A, Neitsch J, Johann T, Hammad S, Hengstler JG, Drasdo D, et al. TiQuant: software for tissue analysis, quantification and surface reconstruction. *Bioinformatics.* 2015;31:3234-6.
- Ghallab A. Highlight report: Acetaminophen hepatotoxicity. *Arch Toxicol.* 2015a;89:2449-51.
- Ghallab A. Interspecies extrapolation by physiologically based pharmacokinetic modeling. *EXCLI J.* 2015b;14:1261-3.
- Ghallab A. Highlight report: New methods for quantification of bile dynamics. *EXCLI J.* 2015c;14:1264-6.
- Ghallab A, Cellière G, Henkel SG, Driesch D, Hoehme S, Hofmann U, et al. Model-guided identification of a therapeutic strategy to reduce in liver diseases. *J Hepatol.* 2016;64:860-71.
- Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S, et al. 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.* 2013;87:1315-530.
- Godoy P, Widera A, Schmidt-Heck W, Campos G, Meyer C, Cadenas C, et al. Gene network activity in cultivated primary hepatocytes is highly similar to diseased mammalian liver tissue. *Arch Toxicol.* 2016;90:2513-29.
- Gómez-Lechón MJ, Tolosa L. Human hepatocytes derived from pluripotent stem cells: a promising cell model for drug hepatotoxicity screening. *Arch Toxicol.* 2016;90:2049-61.
- Grinberg M, Stöber RM, Edlund K, Rempel E, Godoy P, Reif R, et al. Toxicogenomics directory of chemically exposed human hepatocytes. *Arch Toxicol.* 2014;88:2261-87.
- Hammad S, Hoehme S, Friebel A, von Recklinghausen I, Othman A, Begher-Tibbe B, et al. Protocols for staining of bile canalicular and sinusoidal networks of human, mouse and pig livers, three-dimensional reconstruction and quantification of tissue microarchitecture by image processing and analysis. *Arch Toxicol.* 2014;88:1161-83.
- Hassan R. Possibilities and limitations of intravital imaging. *EXCLI J.* 2016;15:872-4.
- Hewitt NJ, Lechón MJ, Houston JB, Hallifax D, Brown HS, Maurel P, et al. 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.* 2007;39:159-234.
- Jansen PL, Ghallab A, Vartak N, Reif R, Schaap FG, Hampe J, et al. The ascending pathophysiology of cholestatic liver disease. *Hepatology.* 2017;65:722-38.
- Kyriakides M, Maitre L, Stamper BD, Mohar I, Kavanagh TJ, Foster J, et al. Comparative analysis of hepatotoxicity induced by acetaminophen and its less toxic meta-isomer. *Arch Toxicol.* 2016;90:3073-85.
- Ramachandran A, Lebofsky M, Yan HM, Weinman SA, Jaeschke H. Hepatitis C virus structural proteins can exacerbate or ameliorate acetaminophen-induced liver injury in mice. *Arch Toxicol.* 2015;89:773-83.
- Ramirez T, Strigun A, Verlohner A, Huener, HA, Peter E, Herold M, et al. Prediction of liver toxicity and mode of action using metabolomics in vitro in HepG2 cells. *Arch Toxicol.* 2017. [Epub ahead of print].
- Reif R, Karlsson J, Günther G, Beattie L, Wrangborg D, Hammad S, et al. Bile dynamics in hepatocyte sandwich cultures. *Arch Toxicol.* 2015;89:1861-70.
- Reif R, Ghallab A, Beattie L, Günther G, Kuepfer L, Kaye PM, et al. In vivo imaging of systemic transport and elimination of xenobiotics and endogenous molecules in mice. *Arch Toxicol.* 2017;91:1335-52.
- Stöber R. Drug-induced mitochondrial impairment in liver cells. *EXCLI J.* 2015;14:1297-9.
- Thiel C, Schneckener S, Krauss M, Ghallab A, Hofmann U, Kanacher T, et al. A systematic evaluation of the use of physiologically based pharmacokinetic modeling for cross-species extrapolation. *J Pharm Sci.* 2015;104:191-206.

Tolosa L, Gómez-Lechón MJ, Donato MT. High-content screening technology for studying drug-induced hepatotoxicity in cell models. *Arch Toxicol.* 2015;89:1007-22.

Vartak N, Damle-Vartak A, Richter B, Dirsch O, Dahmen U, Hammad S, et al. Cholestasis-induced adaptive remodeling of interlobular bile ducts. *Hepatology.* 2016;63:951-64.