LETTER TO THE EDITOR

Response to Pathophysiological Relevance of Proteomics Investigations of Drug-Induced Hepatotoxicity in HepG2 Cells

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In a recent study, we investigated the protein expression changes in HepG2 cells exposed to three well-characterized model compounds (acetaminophen [APAP], amiodarone, and cyclosporin A) (Van Summeren et al., 2011). In a Letter to the Editor, Jaeschke et al. gave their comments on our study, especially with respect to the suitability of HepG2 cells to study liver toxicity, thereby limiting their comments to effects induced by APAP. We are pleased that our research opens the discussion on the use of HepG2 cells and omics technologies in toxicity screening.

The major aim of our research program is to develop in vitro methods or short-term in vivo methods for testing the organotoxic properties of compounds as an alternative to the chronic rodent toxicity assays for long-term toxicity. For that, we consider several in vitro models, namely, HepG2 cells (Staal et al., 2006; Van Summeren et al., 2011), HepaRG cells (Jennen et al., 2010), as well as mouse, rat, and human primary hepatocytes (Kienhuis et al., 2009a; Mathijs et al., 2009).

Ideally, in vitro models should represent in vivo conditions as good as possible; in predictive toxicology, it is emphasized that cellular models should be metabolic competent, thus express relevant phase I and II enzymes (although it is fair to note that the eventual toxic outcome is determined by a much wider range of molecular mechanisms involved). Metabolic competent, however, means that the enzymes should be expressed at a certain level so that they can activate xenobiotic compounds; it does not necessarily imply that enzymatic activities should be similar to in vivo.

In contrast to the statement of Jaeschke et al., it has been shown that HepG2 cells are able to metabolize xenobiotic compounds leading to toxic effects, including genotoxicity oxidative stress and mitochondrial dysfunction (Hewitt and Hewitt, 2004; Knasmuller et al., 2004; O’Brien and Haskins, 2007; Schoonen et al., 2009). However, it is undeniable that the HepG2 cells have lost some of the liver-specific functions due to immortalization, in particular the phase I drug-metabolizing enzymes such as CYP2E1 (Boess et al., 2003; Wilkening and Bader, 2003).

Despite that HepG2 is a carcinoma cell line, it has an epithelial-like morphology, which resembles liver parenchymal cells and synthesizes and secretes a considerable amount of plasma proteins (Knowles et al., 1980; Slany et al., 2010). HepG2 cells are not limited to their availability and have much less biological variation than human primary hepatocytes. Compared with HepaRG, a commercially available more metabolic competent human hepatoma cell line, HepG2 are readily available for every user and do not need to be purchased for each experiment from a single provider. Probably, because of all these reasons, HepG2 is still the most frequently used liver-based in vitro model for toxicogenomics and high-throughput toxicity screening studies (Buczynski et al., 2000; Choi et al., 2010; Cosgrove et al., 2009; Hockley et al., 2009; Hong et al., 2003; Delft et al., 2004; Westerink and Schoonen, 2007; Westerink et al., 2010) and many more.

As described by Jaeschke et al., APAP toxicity requires metabolic activation to a reactive metabolite, N-acetyl-p-benzoquinoneimine. In humans, CYP3A4 is probably the most important P450 at therapeutic concentrations, whereas CYP2E1 and CYP1A2 becoming significantly involved at high plasma levels and at serious intoxication, respectively (Bessem and Vermeulen, 2001). Although CYP2E1 is low expressed in HepG2 cells compared with HepaRG and human primary hepatocytes as highlighted by Jaeschke et al., expressions of CYP1A2 and CYP3A4 are comparable between both hepatoma cell lines (Hart et al., 2010). Apparently, these expressions are sufficient to induce cytotoxicity by APAP in HepG2 cells, resulting in a 20% inhibitory concentration 0.5mM for 72 h exposure (Van Summeren et al., 2011). This was measured by the 3-(4,5-Dimethylthiazol-2-yl)-2,
5-diphenyltetrazolium bromide assay, a test that measures mitochondrial activity and that is widely used to quantify cytotoxicity (Borenfreund et al., 1988; Hamid et al., 2004; Mosmann, 1983). Others, however, could not measure cytotoxicity by APAP in HepG2, such as (McGill et al., 2011) for 24 h exposure or only at a high—10mM—dose (Schoonen et al., 2009).

HepG2 cells appear more sensitive for APAP-induced cytotoxicity than other more metabolic competent human liver cell models. In HepaRG cells, APAP-induced cytotoxicity was observed at 5mM and higher after 24 h exposure (McGill et al., 2011). Noteworthy, also in sandwich-cultured primary hepatocytes, APAP-induced cytotoxicity is limited, with 10mM being the IC10 or IC20 for human and rat hepatocytes, respectively (Kienhuis et al., 2009b), and 1 and 10mM for mouse hepatocytes at 24 and 48 h exposure (Van Summeren, Renes, Lizarraga, Bouwman, Noben, van Delft, Kleinjans, and Mariman, unpublished data).

Taking all these into account, we believe that HepG2 cells are a suitable model to study APAP-induced hepatotoxicity. In addition, we stress that we succeeded in identifying modes of action of the cholestatic compound cyclosporine A in our HepG2 model, where a differential protein expression pattern related to cholestasis was detected. We therefore suggest that Jaeschke et al. are overstating where they comment “How can we expect to obtain drug toxicity data relevant for animals or humans if we use cell lines that have almost nothing in common with a primary hepatocyte in the intact liver.”

Lastly, Jaeschke et al. question “the extreme focus on the latest analytical technology.” Recent omics techniques have already proven their usefulness in toxicology, in particular improving the current in vitro toxicity tests (Amacher, 2010; Blomme et al., 2009). Application of multiple omics techniques have opened a new venue for in-depth exploring biological interpretation of the studied model. In the end, it all comes down to understanding biology.

REFERENCES


