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Cytochrome P450-derived versus Mitochondrial Oxidant Stress in Acetaminophen Hepatotoxicity

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Abstract

In evaluating the mechanisms of acetaminophen hepatotoxicity in experimental systems, it is critical to keep in mind the relevance of the model system for humans. Important aspects of the human toxicity include formation of a reactive metabolite by the cytochrome P450 system and protein adduct formation, which is thought to trigger mitochondrial dysfunction and oxidant stress ultimately causing necrotic cell death. If models that miss critical parts of this well-established mechanism are used, the relevance of the new information for the human toxicity has to be questioned. Therefore, we feel it is necessary to express our concern regarding the recent publication by Jiang et al. (2015).

Keywords

Acetaminophen; Hepatotoxicity; Oxidant Stress; Mitochondria; Protein Adducts; HepG2 cells

To the Editor

We read with interest a recent paper published by Jiang et al. (2015) in which the authors exposed the human hepatoma cell line HepG2 to various doses of acetaminophen (APAP) and measured mitochondrial oxidant stress and gene expression changes. The rationale behind this investigation, according to the authors, was that HepG2 cells do not express CYP2E1 but other APAP-metabolizing CYPs such as CYP1A2 and CYP3A4. The authors argue that because of the low levels of CYP2E1, there are “minimal levels of CYP2E1-induced reactive oxygen species (ROS) formation, which offers the opportunity to explore mitochondrial oxidative liver injury directly induced by APAP. This may provide insight in the toxic mechanisms in individuals with relatively low CYP2E1 expression and enzyme activity.”

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CONFLICT OF INTEREST

None declared

We will focus our comments mainly on two major problems with this manuscript. First, what is the source of ROS formation during APAP hepatotoxicity? The authors invoke an old hypothesis that the oxidant stress in this model is caused by P450-mediated metabolism of APAP. This hypothesis was popular around 1980 when it was recognized that drug metabolism in microsomes can generate ROS (Kuthan and Ullrich, 1982). Wendel and coworkers demonstrated that APAP-induced lipid peroxidation and liver injury can be eliminated by P450 inhibitors and concluded that the P450-mediated drug metabolism was the source of the oxidant stress (Wendel and Feuerstein, 1981). However, Adams et al. (1983) showed in a rat model that there was no increased glutathione disulfide (GSSG) formation, an indicator of ROS, during APAP metabolism. This observation was subsequently confirmed in a mouse model (Smith and Jaeschke, 1989) and later in primary mouse hepatocytes (Bajt et al., 2004). Furthermore, oxidant stress in metabolically competent human HepaRG cells is detectable only after protein adducts formation at later time points after drug metabolism (McGill et al., 2011). Another argument against a drug metabolism-induced oxidant stress is the fact that an APAP overdose in rats causes GSH depletion and even some protein adducts formation but no oxidant stress (McGill et al., 2012b). In contrast, we could clearly demonstrate that high levels of GSSG selectively accumulate in mitochondria only after the drug metabolism phase (Jaeschke, 1990). This was also supported by our finding of selective peroxynitrite formation in mitochondria (Cover et al., 2005). The relevance of the mitochondrial oxidant stress for the mechanism of APAP-induced cell death was further confirmed by the increased injury in Sod2-deficient mice (Ramachandran et al., 2011), the peroxynitrite-mediated inactivation of Sod2 (Agarwal et al., 2011) and the importance of mitochondrial translocation of iron in the mechanism of mitochondrial dysfunction (Kon et al., 2010). Together, these data and many more (reviewed in Jaeschke et al., 2012), do not support the old hypothesis that P450-mediated drug metabolism is a relevant source of oxidant stress during APAP hepatotoxicity *in vivo* or in cultured hepatocytes. In contrast, there is overwhelming evidence that the main source of oxidant stress and peroxynitrite is the mitochondria, which requires P450-mediated reactive metabolite formation and protein adducts to initiate this oxidant stress.

Second, Jiang et al. (2015) argue that HepG2 cells have low CYP2E1 levels but similar CYP1A2 and CYP3A4 levels as HepaRG cells. This argument is highly questionable because it has been shown that HepaRG cells actually have low CYP2E1 levels compared with freshly isolated human hepatocytes and much higher levels of 1A2 and 3A4 than HepG2 cells (Aninat et al., 2005; Guillouzo et al., 2007; Kajsa et al., 2008). In contrast to HepG2 cells, HepaRG cells show extensive GSH depletion, protein adduct formation, mitochondrial dysfunction and cell necrosis (McGill et al., 2011). In fact, the mitochondrial oxidant stress in HepG2 cells reported by the authors is roughly 20% above control cells and the ATP depletion is less than 20% at 48-72 h (Jiang et al., 2015). This compares with a >1,000% increase in oxidant stress in primary mouse hepatocytes within 3-6 hours (Bajt et al., 2004) and an >80% decline of the mitochondrial membrane potential before cell necrosis (Bajt et al., 2004; McGill et al., 2011; Xie et al., 2014). Thus, compared to the quantitative changes observed in primary mouse or human hepatocytes and metabolically competent HepaRG cells, the mitochondrial dysfunction and oxidant stress in HepG2 is minimal at best and is likely irrelevant for the pathophysiology of APAP hepatotoxicity in humans. Case in

point, APAP hepatotoxicity in humans (McGill et al., 2012a) and in primary human hepatocytes (Xie et al., 2014) is characterized by extensive mitochondrial damage. Interestingly, despite substantial mechanistic similarities between primary mouse or human hepatocytes and the metabolically competent hepatoma cell line HepaRG, there are still significant differences, (e.g. the extent and relevance of c-jun N-terminal kinase activation) between these primary cells and the hepatoma cell line (Xie et al., 2014). Therefore, despite the convenience factor of working with these hepatoma cell lines, especially HepG2 cells, mechanistic data on drug toxicity obtained with these cancer cells have to be interpreted with caution and any extrapolation to humans should be avoided until verification can be obtained with primary cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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