LETTER TO THE EDITOR

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

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We read with interest a recent paper by Van Summeren et al. (2011), who reported protein expression changes in HepG2 cells after exposure to various model drugs for 72 h. According to the authors, the objective of this investigation was to explore “new screening methods that address toxicological hazards early in the drug discovery process” and to use “proteomics techniques to gain further insight into the mechanistic processes of the hepatotoxic compounds.” The authors used three compounds (amiodarone, cyclosporine A, and acetaminophen) in their studies. However, we would like to focus our comments on acetaminophen (APAP) because it is a clinically relevant hepatotoxicant and it is the most frequently used model toxin in vivo and in vitro.

Acetaminophen toxicity has been studied extensively during the last 3 decades, and many mechanistic details have been controversially discussed (Jaeschke and Bajt, 2006). However, one aspect is clearly undisputed: APAP toxicity requires metabolic activation to a reactive metabolite (N-acetyl-p-benzoquinone imine, NAPQI), which initially depletes glutathione and then binds to proteins (Nelson, 1990). There is no toxicity without protein binding (Nelson, 1990). Protein adducts have been reported for mouse livers in vivo using various methods (Jollow et al., 1973; Muldrew et al., 2002), a human hepatocyte cell line (HepaRG) (McGill et al., 2011b), and human overdose patients (Davern et al., 2006). In addition, APAP-induced mitochondrial dysfunction, which correlates with mitochondrial protein adduct formation, has been observed in mice (Jaeschke and Bajt, 2006), human HepaRG cells (McGill et al., 2011b), and there is emerging evidence for this mechanism in human overdose patients (McGill et al., 2011a). Thus, in order to gain further insight into the mechanisms of APAP toxicity as the authors intended with their study (Van Summeren et al., 2011), the experimental system has to have the capability to reproduce the basic feature of being able to metabolically activate this drug. However, HepG2 cells, as many other hepatoma cells, have very low levels of P450 enzymes, and therefore, APAP cannot induce relevant toxicity in these cells unless P450 levels are transcriptionally induced (Roe et al., 1993) or the cytochrome P4502E1 gene is transfected into the cells (Dai and Cederbaum, 1995). Though the authors mention this caveat, they justify use of the HepG2 cells by quoting studies by Schoonen et al. (2005a, 2005b), which “classified 70% of the compounds with known toxicity as cytotoxic when using HepG2 cells.” However, it is interesting to note that in both these papers acetaminophen (paracetamol) was classified as “nontoxic” (Schoonen et al. 2005a, 2005b), and in fact, APAP did not produce significant changes in either reactive oxygen species production or depletion of glutathione in HepG2 cells (Schoonen et al., 2005b). In support of the limited metabolic activation in HepG2 cells, protein adduct formation is less than 10% of adducts observed in HepaRG cells even after 24 h with APAP (McGill et al., 2011b). In addition, HepG2 cells do not experience the characteristic mitochondrial dysfunction observed in mice in vivo (Jaeschke and Bajt, 2006), primary mouse hepatocytes (Bajt et al., 2004) and in HepaRG cells (McGill et al., 2011b). The authors’ mention of the expression of Phase II enzymes in HepG2 cells has little relevance here. There are exceptional cases of drug activation or toxification after sulfation, glucuronidation, or glutathionylation (e.g., morphine-6-glucuronide, formaldehyde formation after dichloromethane glutathionylation, etc.). In the case of APAP and most other drugs, however, these mechanisms make up the primary physiological strategy for drug inactivation and elimination. If anything, the expression of these enzymes should further reduce the toxicity of APAP in HepG2 cells.

Nevertheless, hepatoma cells can respond to APAP. Prolonged APAP exposure in these cells leads to caspase-dependent apoptosis (Boulares et al., 2002). In striking contrast, all cells that are capable of metabolically activating APAP do not show evidence of caspase activation or apoptosis.
be the main reason why there is little use for data generated by many of these “omics” studies.

REFERENCES


