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EDITORIAL

# Highlight report: acetaminophen hepatotoxicity

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Acetaminophen (APAP) represents the leading cause of liver failure in Europe (Lancaster et al. 2015). Much effort has been invested to gain a better understanding of the molecular mechanisms of APAP hepatotoxicity (Saito et al. 2010; Kon et al. 2004; Hwang et al. 2015; Sjogren et al. 2014; Schyschka et al. 2013; Singh et al. 2013) and to identify biomarkers of APAP overdose (McGill et al. 2014; Beger et al. 2015). APAP is known to be metabolically activated to the reactive N-acetyl-p-benzoquinone imine (NAPQI), which forms protein adducts including mitochondrial proteins leading to mitochondrial oxidative stress (Ramachandran et al. 2013; Cohen et al. 1997). As a consequence c-jun N-terminal kinase is translocated to the mitochondria, which enhances generation of reactive oxygen species (Ramachandran et al. 2013; Hanawa et al. 2008; Saito et al. 2010). This may lead to opening of the mitochondrial membrane transition pore and cause a form of cell death which differs from classical apoptosis and has been named 'necroptosis' (Kon et al. 2004; Gujral et al. 2002; Ni et al. 2012). Although cytochrome c is released from the mitochondria, for so far unknown reasons apoptotic cell death is not induced (Gujral et al. 2002; Lawson et al. 1999; Williams et al. 2010). In a recent issue of the Archives of Toxicology, Lancaster and colleagues have published an updated review on diagnosis and clinical management of APAP-induced liver failure (Lancaster et al. 2015). One of the most urgent clinical research fields seems to be the identification of biomarkers that predict more reliably whether a patient is in need of treatment and hospitalization. Lancaster and colleagues discuss a case in which a patient died, who did not receive *N*-acetylcysteine according to the '200 µg/ml line'. Therefore, it became standard in the UK to treat any patient with serum APAP equivalent to only 100 µg/ml at 4 h after intoxication (Lancaster et al. 2015). However, because the majority of patients with an overdose do not show such serve consequences, the new UK standard means that one life is saved every 2 years associated with costs of 17.3 million euros per saved life. This underlines the urgent need of better predictive biomarkers (Lancaster et al. 2015).

The article of Lancaster et al. (2015) is also of relevance for the field of alternative methods (Hammad 2013; Ghallab 2013, 2014a, b; Reif 2014a, b; Godoy 2011). Currently, intensive research activities are ongoing to establish in vitro systems particularly for hepatotoxicity (Grinberg et al. 2014; Ramaiahgari et al. 2014; Rodrigues et al. 2013; Schug et al. 2013), nephrotoxicity (Sanchez-Niño et al. 2014; Fujiki et al. 2013; 2014), neurotoxicity (Barbosa et al. 2014; Sisnaiske et al. 2014; Frimat et al. 2010) and developmental toxicity (Krug et al. 2013; Rempel et al. 2015; Balmer et al. 2014; Zimmer et al. 2014). For validation of in vitro systems with hepatocytes APAP represents a popular reference compound (Jennings et al. 2014; Hengstler et al. 2014). However, it can often be observed that in vitro studies used very high APAP concentrations in the range of 10 mM or even higher. The review of Lancaster et al. (2015) reminds us of the time-honored '200 µg/ml line', which means that a serum concentration of 200 µg APAP/ml 4 h after oral uptake represents a threshold above which the risk of acute liver failure significantly increases. Considering the molecular weight of APAP of 151.16 g/mol this corresponds to a threshold of 1.3 mM. It should also be considered that due to a half-life of 1–4 h in human, concentrations in vivo decrease much faster than in vitro.

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Most likely the reason for resistance of cultivated hepatocytes to APAP is decreased expression CYP2E1 and further cytochrome P450 enzymes involved in APAP metabolic activation. It is well known that the isolation and cultivation stress lead to a rapid decrease in metabolizing enzymes in human hepatocytes (Godoy et al. 2013, 2015) and even more in rodents (Zellmer et al. 2010; Heise et al. 2012; Godoy et al. 2009). Because of the relatively large discrepancy between APAP concentrations in vivo and higher concentrations chosen in many in vitro studies, it should be interpreted with caution, whether mechanisms observed in vitro at 10 mM and higher actually represent the in vivo situation.

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