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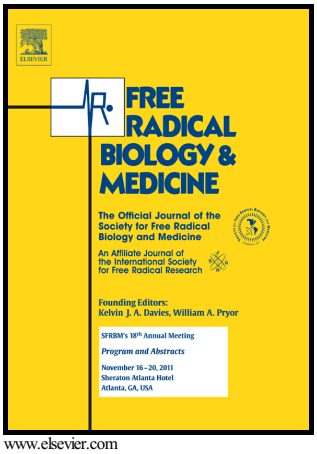

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Endoplasmic reticulum stress precedes oxidative stress in antibiotic-induced cholestasis and cytotoxicity in human hepatocytes

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Abstract

Endoplasmic reticulum (ER) stress has been associated with various drug–induced liver lesions but its participation in drug-induced cholestasis remains unclear. We first aimed at analyzing liver damage caused by various hepatotoxic antibiotics, including three penicillinase-resistant antibiotics (PRAs), i.e. flucloxacillin, cloxacillin and nafcillin, as well as trovafloxacin, levofloxacin and erythromycin, using human differentiated HepaRG cells and primary hepatocytes. All these antibiotics caused early cholestatic effects typified by bile canaliculi dilatation and reduced bile acid efflux within 2h and dose-dependent enhanced caspase-3 activity within 24h. PRAs induced the highest cholestatic effects at non cytotoxic concentrations. Then, molecular events involved in these lesions were analyzed. Early accumulation of misfolded proteins revealed by thioflavin-T fluorescence and associated with phosphorylation of the unfolded protein response sensors, eIF2α and/or IRE1α, was evidenced with all tested hepatotoxic antibiotics. Inhibition of ER stress markedly restored bile acid efflux and prevented bile canaliculi dilatation. Downstream of ER stress, ROS were also generated with high antibiotic concentrations. The protective HSP27-PI3K-AKT signaling pathway was activated only in PRA-treated cells and its inhibition increased ROS production and aggravated caspase-3 activity. Overall, our results demonstrate that (i) various antibiotics reported to cause cholestasis and hepatocellular injury in the clinic can also induce such effects in *in vitro* human hepatocytes; (ii) PRAs cause the strongest cholestatic effects in the absence of cytotoxicity; (iii) cholestatic features occur early through ER stress; (iv) cytotoxic lesions are observed later

[1] Both authors contributed equally to this work.