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Alcohol-induced polar retinoid metabolites trigger hepatocyte apoptosis via loss of mitochondrial membrane potential.

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Chronic alcohol consumption depletes hepatic vitamin A stores. However, vitamin A supplementation may be hepatotoxic which is further potentiated by concomitant alcohol consumption. The mechanisms for this phenomenon are poorly understood. Recently, it was suggested that polar retinol metabolites generated by alcohol-inducible cytochrome P4502E1 might aggravate liver damage. However, direct experimental evidence supporting this hypothesis is still lacking.

In the present study, we investigated the toxic effects of PRMs in cultured HepG2 cells and primary rat hepatocytes and particularly emphasized in the pathogenesis of PRMs-induced toxicity of hepatocytes. PRMs were extracted from liver tissues of Sprague-Dawley rats fed either an alcoholic or isocaloric control Lieber-DeCarli liquid diet for one month by HPLC. We determined the cell toxicity by BrdU incorporation assay, morphological assessment, trypan blue exclusion test, and LDH/AST leakage measurements. Staining for DAPI and AO, FACS analysis and Western Immunoblotting for cleaved caspase-9 and caspase-3 were used to detect apoptosis. Our studies yielded the following results: PRMs caused marked cytotoxicity in a concentration- and time-dependent manner in both cell types reflected by morphological changes, a dramatic increase in trypan blue positive cells and LDH/AST leakage. This toxicity was due to apoptosis, as demonstrated by a time-dependent increase of sub-G1 cellular events, a rapid loss of mitochondrial membrane potential ($\Delta\Psi m$) and a time-dependent activation of caspase-9 and caspase-3. No toxicity was found with equivalent dilution of the control extracts from non-alcoholic rats.

We proposed a model of the possible mechanism of PRMs-induced apoptosis in hepatocytes that summarizes all our experimental data. Chronic ethanol comsumption leads to an induction of CYP2E1 activity. CYP2E1 oxidizes ROH and RA into PRMs. As a result, PRMs trigger the reduction of $\Delta\Psi m$. Subsequently, apoptosis-inducing factors (cyto c) are released. Released cyto c activates caspase-9, followed by activation of caspase-3 and initiation of the proteolytic cascade that culminates in apoptosis.

In summary, our present data demonstrate for the first time that PRMs causes cell death in HepG2 cells and isolated rats hepatocytes. Cytotoxicity is mainly due to apoptosis and is initiated by the loss of $\Delta\Psi m$ followed by caspases activation. These data provide a mechanistic explanation for the potentiation of vitamin A hepatotoxicity by alcohol and close the loop between the induction of CYP2E1, the production of increased amounts of PRMs, and resulting liver damage.