HEPATOLOGY

HEPATOLOGY, VOL. 69, NO. 2, 2019

Bile Microinfarcts in Cholestasis Are Initiated by Rupture of the Apical Hepatocyte Membrane and Cause Shunting of Bile to Sinusoidal Blood

Ahmed Ghallab ,[1,2] Ute Hofmann,[3] Selahaddin Sezgin,[4] Nachiket Vartak,[1] Reham Hassan,[1,2] Ayham Zaza,[1,5] Patricio Godoy,[1] Kai Markus Schneider,[6] Georgia Guenther,[1] Yasser A. Ahmed,[7] Aya A. Abbas,[2] Verena Keitel,[8] Lars Kuepfer,[9] Steven Dooley,[10] Frank Lammert,[11] Christian Trautwein,[6] Michael Spiteller,[4] Dirk Drasdo,[1,5] Alan F. Hofmann,[12] Peter L.M. Jansen,[13*] Jan G. Hengstler,[1*] and Raymond Reif[1*]

Bile duct ligation (BDL) is an experimental procedure that mimics obstructive cholestatic disease. One of the early consequences of BDL in rodents is the appearance of so-called bile infarcts that correspond to Charcot-Gombault necrosis in human cholestasis. The mechanisms causing bile infarcts and their pathophysiological relevance are unclear. Therefore, intravital two photon–based imaging of BDL mice was performed with fluorescent bile salts (BS) and non-BS organic anion analogues. Key findings were followed up by matrix-assisted laser desorption ionization imaging, clinical chemistry, immunostaining, and gene expression analyses. In the acute phase, 1-3 days after BDL, BS concentrations in bile increased and single-cell bile microinfarcts occurred in dispersed hepatocytes throughout the liver caused by the rupture of the apical hepatocyte membrane. This rupture occurred after loss of mitochondrial membrane potential, followed by entry of bile, cell death, and a "domino effect" of further death events of neighboring hepatocytes. Bile infarcts provided a trans-epithelial shunt between bile canaliculi and sinusoids by which bile constituents leaked into blood. In the chronic phase, ≥21 days after BDL, uptake of BS tracers at the sinusoidal hepatocyte membrane was reduced. This contributes to elevated concentrations of BS in blood and decreased concentrations in the biliary tract. Co*nclusion:* Bile microinfarcts occur in the acute phase after BDL in a limited number of dispersed hepatocytes followed by larger infarcts involving neighboring hepatocytes, and they allow leakage of bile from the BS-overloaded biliary tract into blood, thereby protecting the liver from BS toxicity; in the chronic phase after BDL, reduced sinusoidal BS uptake is a dominant protective factor, and the kidney contributes to the elimination of BS until cholemic nephropathy sets in. (Hepatology 2019;69:666-683).

SEE EDITORIAL ON PAGE 473

Recently, the concept of an ascending pathophysiology of cholestatic liver disease has been proposed, whereby lesions start in large or small bile ducts followed by the involvement of upstream structures, such as the bile canalicular network and liver parenchyma.[1] Our knowledge of the responses of the biliary tree to cholestasis stems mainly from studies in rodents after bile duct ligation

Abbreviations: Abcb11, adenosine triphosphate–binding cassette, subfamily B, member 11; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; ATP, adenosine triphosphate; BDL, bile duct ligation; BS, bile salts; CD45, cluster of differentiation 45; CDCA, chenodeoxycholic acid; CK-19, cytokeratin-19; CLF, cholyl-lysyl-fluorescein; CMF, 5-chloromethylfluorescein; CMFDA, CMF diacetate; Ly6G, lymphocyte antigen 6 complex locus G; MALDI, matrix-assisted laser desorption ionization; Mdr2, multidrug resistance gene 2; MRP, multidrug resistance–associated protein; Ntcp, Na^+-taurocholate cotransporting polypeptide; OATP, organic ion transporting polypeptide; PI, propidium iodide; Slc10a1, solute carrier family 10, member a1; TMRE, tetramethylrhodamine ethyl ester.

Received October 21, 2017; accepted July 20, 2018.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.30213/suppinfo.

**These authors contributed equally to this work.*

Supported by the Bundesministerium für Bildung und Forschung (BMBF, Germany)–funded project LiSyM (FKZ 031L0052, 031L0045, 031L0055, 031L0037, 031L0051), including contributions from the following projects: BMBF (031L0073F, 031A355A), IMI (031L0117B), and an AOP concept of EUToxRisk (681002).