Two-photon based imaging reveals mechanisms of tissue damage

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In the recent issue of *Hepatology*, Ahmed Ghallab and colleagues from Dortmund University published an article, where they visualize the key event leading to bile infarcts, rupture of the apical hepatocyte membrane in individual hepatocytes of cholestatic livers (Ghallab et al. 2018). A central technique in this study is intravital two-photon microscopy. This technique allows imaging of intact organs in living anaesthetized mice. Recently, the method has been optimized by a well-matched combination of infrared lasers, specific long-distance objectives with high numerical aperture and very sensitive GaAsP detectors (Reif et al. 2017; Köppert et al. 2018). This allows resolution close to the theoretically possible 200 nm. Therefore, bile canaliculi which have diameters ranging between 500 and 2000 nm (Hammad et al. 2014) can be visualized by this method. Similarly important is that fast sequences in the millisecond range can be recorded. Therefore, it was possible for the first time to record the fast process of apical membrane rupture. Initially, the local bile canaliculus swells and then suddenly ruptures, leading to regurgitation of the toxic canalicular bile acids into the adjacent hepatocyte. Interestingly, this isolated event with only one ruptured hepatocyte leads to a kind of domino effect where neighboring hepatocytes are also affected, leading to a growing necrotic region that accumulates bile acids, the so-called ‘bile infarct’. This bile infarct differs from infarcts in other organs, where a localized area of dead tissue has been deprived of its blood supply; in liver, bile infarcts are not formed because of limited oxygen but due to bile leakage. The intravital imaging of Ghallab et al. (2018) now elucidates how exactly this pathological process is triggered.

Cholestasis represents an intensively studied field of research in toxicological sciences (Starokozhko et al. 2017; Mischczuk et al. 2015; Toledo et al. 2017; Vinken et al. 2017;

Leist et al. 2017). The biliary tract has been shown to consist of three anatomical domains that respond differently to cholestasis (Vartak et al. 2016): large ducts widen their diameters, interlobular ducts respond by branching and looping thereby forming a denser mesh, while the most upstream domain, the bile canaliculi, become wider and form spiny protrusions into hepatocytes (Vartak et al. 2016; Jansen et al. 2017). Much effort has been invested in developing in vitro test systems for cholestasis (Deharde et al. 2016; Luckert et al. 2017; Frey et al. 2014; Godoy et al. 2013; Reif et al. 2015) and transcriptomic signatures have been identified (Parmentier et al. 2017; Ghallab 2017; Stöber 2016; Grinberg et al. 2014). However, so far the observation of Ghallab et al. (2018) that rupture of apical hepatocyte membranes is a key event, causing bile infarcts has only been possible to be demonstrated in vivo.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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