

Guest editorial:

HIGHLIGHT REPORT: NEW INSIGHTS IN LIVER PHYSIOLOGY: CANALICULAR BILE FLUX IS DIFFUSION DOMINATED

Ahmed Ezzat Ahmed

Department of Biology, College of Science, King Khalid University, 61413 Abha, Asir,
Saudi Arabia, E-mail: Aabdelrahman@kku.edu.sa

<http://dx.doi.org/10.17179/excli2020-2836>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>).

One of the central functions of the liver is excretion of bile into the intestine. Currently, bile excretion is explained by the osmotic model, according to which bile acids are excreted by hepatocytes into the bile canaliculi and since bile acids are osmotically active they draw water into the canalicular lumen. Bile canaliculi are closed at the central side. Therefore, bile was postulated to flow to the open side into the ducts. However, bile flow in canaliculi has never been measured because of the small canalicular diameter which does not allow analysis of flux by conventional methods. Recently, methods have been developed that allow flow analysis in bile canaliculi and ducts. Interestingly, no measurable directed flow was observed in the canaliculi. Instead, small molecules in bile canaliculi reached the larger bile ducts by diffusion. Only there measurable flow sets in. The pathophysiological implications of this novel observation are discussed.

In the current issue of *Hepatology* a novel concept has been published how the liver transports bile through canaliculi and ducts to the intestine (Vartak et al., 2020). Hepatocytes are known to excrete bile acids and xenobiotics into a ‘canal system’ that finally drains into the intestine (Godoy et al., 2013). The most upstream part of this canal system, the bile canaliculi, are lined by the membrane of hepatocytes. With only 0.5-2 μm diameter,

bile canaliculi represent very thin vessels. They are connected to interlobular bile ducts by a connecting pipe, the so-called Hering channel (Vartak et al., 2016). In contrast to bile canaliculi, the bile ducts are lined by epithelial cells, the cholangiocytes. In the present study, the authors show that it is important to differentiate bile canaliculi and bile ducts as functionally distinct domains (Vartak et al., 2020): transport of small molecules in the bile canaliculi is diffusion dominated and only in the ducts diffusion is augmented by flow due to water influx (Vartak et al., 2020). According to this model, bile canaliculi can be compared to a lake with standing water that is connected to a river, the bile duct. When a compound is given into the lake, it will also reach the river – by diffusion – and will only then be carried away by flow. This new model (Vartak et al., 2020) contradicts the prevailing osmotic model of bile flow that is present in medical text books since decades (Boyer, 2013; Boyer and Bloomer, 1974; Boyer et al., 1970; Layden et al., 1978; Sperber, 1959; Wheeler and Ramos, 1960; Wheeler et al., 1968; Wood et al., 1977). According to the osmotic model, bile acids are excreted by hepatocytes into the bile canaliculi. Bile acids are osmotically active and draw water into the canalicular lumen. Since bile canaliculi are closed at their pericentral end, bile should flow to the open side into the ducts. However,

bile flow in canaliculi has never been measured. Because of the small canalicular diameter flow analysis cannot be accomplished by conventional methods. Vartak and colleagues now established a method that allows the quantification of flow and diffusion in bile canaliculi and ducts in intact livers of living mice (Vartak et al., 2020). For this purpose, they used a photoactivatable compound, CMNB-fluorescein, which only upon UV irradiation releases fluorescein that then can be detected. Importantly, CMNB-fluorescein is excreted into bile canaliculi. Using an intravital method, Vartak and colleagues photoactivated CMNB-fluorescein in small tissue regions of intact livers, simultaneously imaging the fluorescence generated in the UV exposed region. The result was unexpected, since the fluorescent material photoactivated in a small region of the canalicular network, spread symmetrically into the surrounding canaliculi, which indicates diffusion rather than flow. In contrast, when photoactivation was performed in a blood vessel, the photoactivated material rapidly moved unidirectionally with the blood flow. A particularly convincing set of data was obtained, when the authors photoactivated CMNB-fluorescein within a Hering channel. As expected, fluorescent material moved downstream into the bile duct; however, unexpectedly also travelled retrograde, upstream into the canalicular network. This retrograde flux would not be possible in a flow dominated system.

In their previous work, the authors studied liver physiology based on mathematical models (Hoehme et al., 2010; Schliess et al., 2014; Bartl et al., 2015; Schenk et al., 2017; Ghallab et al., 2016) and elucidated the microarchitecture of the biliary tract by imaging and 3D reconstruction (Hammad et al., 2014; Damle-Vartak et al., 2019; Friebel et al., 2015). However, later they began to focus on intravital imaging of physiological processes (Reif et al., 2017; Ghallab et al., 2019; Köppert et al., 2018). The present study demonstrates the importance of analyzing physiological parameters in intact organs *in vivo*, because it

may be misleading to exclusively rely on model simulations.

The findings of Vartak and colleagues overturn long-standing assumptions about how the liver excretes bile into the duodenum. The seemingly subtle difference between flow and diffusion becomes relevant, when it comes to identification of adequate therapeutic strategies for liver diseases, such as non-alcoholic fatty liver disease (NAFLD). In some cholestatic liver diseases the bile canalicular network shows alterations, such as limited connectivity to the ducts (Vartak et al., 2016; Jansen et al., 2017). If the same volume of liquid would have to pass the compromised canaliculi by advection, this should result in a build-up of pressure, which could damage liver tissue. Therefore, drugs should be identified that reduce the assumed flow, which would also prevent the increase of damaging pressure. However, based on the present results (Vartak et al., 2020), this flow-pressure concept should be questioned. It will be interesting to observe the further discussion about the correct model of bile flux and its pathophysiological as well as clinical consequences.

Conflict of interest

The author declares that he has no conflict of interest.

REFERENCES

- Bartl M, Pfaff M, Ghallab A, Driesch D, Henkel SG, Hengstler JG, et al. Optimality in the zonation of ammonia detoxification in rodent liver. *Arch Toxicol.* 2015;89:2069-78. doi: 10.1007/s00204-015-1596-4.
- Boyer JL. Bile formation and secretion. *Compr Physiol.* 2013;3:1035-78. doi: 10.1002/cphy.c120027.
- Boyer JL, Bloomer JR. Canalicular bile secretion in man. Studies utilizing the biliary clearance of (14C) mannitol. *J Clin Invest.* 1974;54:773-81. doi: 10.1172/JCI107817.
- Boyer JL, Scheig RL, Klatskin G. The effect of sodium taurocholate on the hepatic metabolism of sulfobromophthalein sodium (BSP). The role of bile flow. *J Clin Invest.* 1970;49:206-15. doi: 10.1172/JCI106229.

- Damle-Vartak A, Begher-Tibbe B, Gunther G, Geisler F, Vartak N, Hengstler JG. Pipe-3D: A pipeline based on immunofluorescence, 3D confocal imaging, reconstructions, and morphometry for biliary network analysis in cholestasis. *Methods Mol Biol.* 2019;1981:25-53. doi: 10.1007/978-1-4939-9420-5_3.
- Friebel A, Neitsch J, Johann T, Hammad S, Hengstler JG, Drasdo D, et al. TiQuant: software for tissue analysis, quantification and surface reconstruction. *Bioinformatics.* 2015;31:3234-6. doi: 10.1093/bioinformatics/btv346.
- Ghallab A, Cellière G, Henkel SG, Driesch D, Hoehme S, Hofmann U, et al. Model-guided identification of a therapeutic strategy to reduce hyperammonemia in liver diseases. *J Hepatol.* 2016;64:860-71. doi: 10.1016/j.jhep.2015.11.018.
- Ghallab A, Hofmann U, Sezgin S, Vartak N, Hassan R, Zaza A, et al. Bile microinfarcts in cholestasis are initiated by rupture of the apical hepatocyte membrane and cause shunting of bile to sinusoidal blood. *Hepatology.* 2019;69:666-83. doi: 10.1002/hep.30213.
- Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S, et al. Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. *Arch Toxicol.* 2013;87:1315-530. doi: 10.1007/s00204-013-1078-5.
- Hammad S, Hoehme S, Friebel A, von Recklinghausen I, Othman A, Begher-Tibbe B, et al. Protocols for staining of bile canalicular and sinusoidal networks of human, mouse and pig livers, three-dimensional reconstruction and quantification of tissue microarchitecture by image processing and analysis. *Arch Toxicol.* 2014; 88:1161-83. doi: 10.1007/s00204-014-1243-5.
- Hoehme S, Brulport M, Bauer A, Bedawy E, Schorrmann W, Hermes M, et al. Prediction and validation of cell alignment along microvessels as order principle to restore tissue architecture in liver regeneration. *Proc Natl Acad Sci U S A.* 2010;107:10371-6. doi: 10.1073/pnas.0909374107.
- Jansen PL, Ghallab A, Vartak N, Reif R, Schaap FG, Hampe J, et al. The ascending pathophysiology of cholestatic liver disease. *Hepatology.* 2017;65:722-38. doi: 10.1002/hep.28965.
- Köppert S, Büscher A, Babler A, Ghallab A, Buhl EM, Latz E, et al. Cellular clearance and biological activity of calciprotein particles depend on their maturation state and crystallinity. *Front Immunol.* 2018;9:1991. doi: 10.3389/fimmu.2018.01991.
- Layden TJ, Elias E, Boyer JL. Bile formation in the rat: the role of the paracellular shunt pathway. *J Clin Invest.* 1978;62:1375-85. doi: 10.1172/JCI109258.
- Reif R, Ghallab A, Beattie L, Günther G, Kuepfer L, Kaye PM, et al. In vivo imaging of systemic transport and elimination of xenobiotics and endogenous molecules in mice. *Arch Toxicol.* 2017;91:1335-52. doi: 10.1007/s00204-016-1906-5.
- Schenk A, Ghallab A, Hofmann U, Hassan R, Schwarz M, Schuppert A, et al. Physiologically-based modeling in mice suggests an aggravated loss of clearance capacity after toxic liver damage. *Sci Rep.* 2017;7: 6224. doi: 10.1038/s41598-017-04574-z.
- Schliess F, Hoehme S, Henkel SG, Ghallab A, Driesch D, Böttger J, et al. Integrated metabolic spatial-temporal model for the prediction of ammonia detoxification during liver damage and regeneration. *Hepatology.* 2014;60:2040-51. doi: 10.1002/hep.27136.
- Sperber I. Secretion of organic anions in the formation of urine and bile. *Pharmacol Rev.* 1959;11:109-34.
- Vartak N, Damle-Vartak A, Richter B, Dirsch O, Dahmen U, Hammad S, et al. Cholestasis-induced adaptive remodeling of interlobular bile ducts. *Hepatology.* 2016;63:951-64. doi: 10.1002/hep.28373.
- Vartak N, Guenther G, Joly F, Damle-Vartak A, Wibbelt G, Fickel J, et al. Intravital dynamic and correlative imaging reveals diffusion-dominated canalicular and flow-augmented ductular bile flux. *Hepatology.* 2020; Jun 19. Online ahead of print. doi: 10.1002/hep.31422.
- Wheeler HO, Ramos OL. Determinants of the flow and composition of bile in the unanesthetized dog during constant infusions of sodium taurocholate. *J Clin Invest.* 1960;39:161-70. doi: 10.1172/JCI104015.
- Wheeler HO, Ross ED, Bradley SE. Canalicular bile production in dogs. *Am J Physiol.* 1968;214:866-74. doi: 10.1152/ajplegacy.1968.214.4.866.
- Wood RA, Baker AL, Hall AW, Boyer JL, Moossa AR. Evaluation of a new monkey model for the repeated study of bile secretory physiology. *Ann Surg.* 1977;185:349-55. doi: 10.1097/00000658-197703000-00017.