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Hepatic Congestion Leads to Fibrosis: Findings in a Newly Developed Murine Model

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Passive hepatic congestion, known as congestive hepatopathy, occurs due to hepatic outflow obstruction, a condition most commonly observed in congestive heart failure. Chronic hepatic congestion can eventually result in hepatic fibrosis. Liver specimens of patients with hepatic congestion are histologically characterized by sinusoidal engorgement and hemorrhagic necrosis in the perivenular areas of the hepatic acini, which leads to sinusoidal fibrosis and ultimately to bridging fibrosis between adjacent central veins.^{1–2} Our understanding of fibrogenesis in congestive hepatopathy has largely come from pathological examinations of human samples. The mechanisms have remained unclear partly due to the lack of appropriate experimental models.

In the current issue of *HEPATOLOGY*, Simonetto et al.³ developed a murine model of congestive hepatopathy through partial ligation of the inferior vena cava (pIVCL). Using this model and *in vitro* cell culture system, they demonstrated that chronic hepatic congestion causes sinusoidal thrombus formation as well as sinusoidal stretch, which both facilitate fibronectin (FN) fibril assembly by hepatic stellate cells (HSCs), an early step in extracellular matrix deposition, and eventually hepatic fibrosis.³ This work is novel and important, particularly due to the development of a relatively easy surgical procedure to generate hepatic congestion in rodents, which allowed them to investigate a mechanistic link between congestive hepatopathy and fibrosis.

In their pIVCL model, the inferior vena cava (IVC) was ligated along with a sterile steel wire of 0.6 mm in diameter. The wire was placed alongside the IVC and the 2 ligated together, with the wire acting as a spacer or placeholder for the ligature. The wire was removed immediately after the ligation, which reduced the IVC diameter by approximately 70%. The authors characterized this model intensively and verified that mice given pIVCL presented pathological features similar to those seen in patients with congestive hepatopathy, including the development of fibrosis. An exception was cardiac output. While

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reduced cardiac output was reported in patients, no reductions were observed in this mouse model.

There were two key observations in their pIVCL model. One was minimal inflammatory activity in the liver despite the development of fibrosis. Given that chronic inflammation has a pivotal role in the majority of fibrotic cases, this was a unique feature and suggested a non-inflammatory mechanism of fibrogenesis. In fact, minimal hepatic inflammation was observed in patients with the Fontan circulation, another cause of cardiac disease-related hepatic congestion, suggesting that fibrosis can develop independently of inflammation in this condition.⁴ The other was sinusoidal thrombus formation, which was evident by the presence of fibrin in liver specimens of pIVCL mice as well as those of patients with congestive hepatopathy. The authors examined if the disruption of the coagulation process by pharmacological (warfarin treatment) and genetic [tissue factor pathway inhibitor (TFPI) overexpressing mice] measures reduces fibrosis in the pIVCL model (Figure 1). TFPI is an endogenous inhibitor of the extrinsic coagulation pathway⁵, and thus mice overexpressing TFPI prevent thrombosis. These two approaches significantly reduced intrahepatic fibrin levels and fibrosis, indicating an effect of thrombosis on fibrogenesis in congestive hepatopathy.

How does thrombus formation contribute to fibrosis? The coagulation process generates mature fibrin clots through cleavage of circulating fibrinogen by the action of thrombin, a potent plasma protease.⁶ Fibrin facilitates binding of fibronectin (FN) to fibrin (FN fibril assembly)⁷, a key process for the development of a provisional extracellular matrix. FN fibril assembly is initiated by binding of FN to integrins, mainly $\alpha 5 \beta 1$ integrin, followed by cytoskeletal rearrangements and Rho-dependent cellular contractions.⁸ The authors verified that fibrin stimulates FN fibril assembly by HSCs in an integrin and actin dependent manner, linking hepatic thrombosis to fibrosis. In addition, they demonstrated that mechanical forces such as sinusoidal stretch could also promote FN fibril assembly (Figure 1). Hepatic congestion could increase intrahepatic pressure (stress), resulting in a stretch of endothelial cells (ECs) and HSCs. The effects of mechanical strain on ECs and HSCs have not been well characterized.

In this study, a series of *in vivo* and *in vitro* experiments were logically designed and thoroughly executed, which led to the demonstration of two new potential mechanisms of fibrogenesis, namely thrombus- and mechanical force-mediated fibrogenesis. However, as every novel study raises questions, so does this one. Several come to mind. What would happen in pIVCL mice, when the postoperation period is extended longer than the current 6 weeks? Given a relatively mild degree of fibrosis at 6 weeks of pIVCL, do mice with pIVCL develop fibrosis further, if they are kept longer? At that time point, would inflammation still not be observed? Would there be development of edema or ascites, which is also a feature of advanced congestive hepatopathy?¹

Given the development of fibrosis in the pericentral and sinusoidal areas in congestive hepatopathy, it may also be interesting to examine the involvement of fibroblasts around the central vein in fibrogenesis. Hemodynamic changes due to hepatic congestion may change local mechanical forces, which could facilitate activation of pericentral fibroblasts as well as

HSCs to profibrotic myofibroblasts⁹ and thereby contribute to ECM deposition. Further, since liver sinusoidal endothelial cells (LSECs) are exposed to hemodynamic changes directly,¹⁰ they may also have a role in this process. In general, the effects of hemodynamic changes and related local mechanical changes on fibrogenesis are not well understood. The pIVCL model will be a good tool for studies in this area.

There may also be an involvement of nitric oxide (NO) in congestive hepatopathy. Sinusoidal stretch due to hepatic congestion may disturb LSEC function. A major feature of EC dysfunction is diminished NO production.¹¹ Endothelial cell-derived NO inhibits platelet aggregation, thereby inhibiting thrombosis.¹² Diminished NO may contribute to increased thrombus formation in congestive hepatopathy. Given the vascular aspects of congenic hepatopathy, the role of NO in this disease would be an area warranting additional investigation.

This study again raises important questions of whether or not anticoagulant therapy has a role in patients with liver fibrosis/cirrhosis as well as in those patients with hepatic congestion. The clotting aspect of fibrosis/cirrhosis has been suggested. Besides the present study, one study reported the efficacy of warfarin in prevention of hepatic fibrogenesis in mice exposed to carbon tetrachloride (CCl₄).¹³ Another study reported that a low-molecular-weight heparin (LMWH) reduced fibrogenesis in rats with CCl₄ treatment.¹⁴ Moreover, efficacy of enoxaparin, an LMWH, was shown for hepatic decompensation as well as the treatment and prevention of portal vein thrombosis in patients with advanced cirrhosis.¹⁵

All these and other questions may be answered in part with studies using this pIVCL model. The pIVCL model will be beneficial for understanding the pathogenesis and pathophysiology of congestive hepatopathy as well as other similar diseases such as veno-occlusive disease and Budd-Chiari syndrome, and thus for developing therapeutic strategies for these diseases. This model will also be useful for understanding the effects of hemodynamic changes in the hepatic circulation on liver pathophysiology in general, which also has significant implications in the liver-heart axis, a well recognized, but under-explored interaction.

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Abbreviations

CCl ₄	carbon tetrachloride
ECs	endothelial cells
ECM	extracellular matrix
FN	fibronectin
HSCs	hepatic stellate cells
IVC	inferior vena cava

LMWH	low-molecular weight-heparin
LSECs	liver sinusoidal endothelial cells
NO	nitric oxide
pIVCL	partial ligation of the inferior vena cava
TFPI	tissue factor pathway inhibitor

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Figure 1. Sinusoidal thrombosis and mechanical strain lead to liver fibrosis in congestive hepatopathy

In the coagulation process, mature fibrin clots are formed through cleavage of circulating fibrinogen by the action of thrombin, a potent plasma protease. Fibrin formation promotes binding of fibronectin (FN) to fibrin (FN fibril assembly), a key process of the development of a provisional extracellular matrix (ECM). FN fibril assembly is initiated by binding of FN to integrins, mainly $\alpha 5 \beta 1$ integrin, which is assembled with intracellular contractile filamentous actin in hepatic stellate cells (HSCs). The assembly of filamentous actin is regulated by bidirectional signaling between Rho kinase activity and integrins. Similarly, mechanical strain due to hepatic congestion promotes FN fibril assembly in the integrin and actin dependent manner.