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Role of Fibrin(ogen) in Progression of Liver Disease: Guilt by Association?

Anna K. Kopec, PhD[1] and **James P. Luyendyk, PhD**[1,2]

[1]Department of Pathobiology & Diagnostic Investigation, Institute for Integrative Toxicology, Michigan State University, East Lansing, Michigan 48824.

[2]Department of Pharmacology and Toxicology, Institute for Integrative Toxicology, Michigan State University, East Lansing, Michigan 48824.

Abstract

Strong experimental evidence indicates that components of the hemostatic system, including thrombin, exacerbate diverse features of experimental liver disease. Clinical studies have also begun to address this connection and some studies have suggested that anticoagulants can improve outcome in patients with liver disease. Amongst the evidence of coagulation cascade activation in models of liver injury and disease is the frequent observation of thrombin-driven hepatic fibrin(ogen) deposition. Indeed, hepatic fibrin(ogen) deposition has long been recognized as a consequence of hepatic injury. Although commonly inferred as pathologic due to protective effects of anticoagulants in mouse models, the role of fibrin(ogen) in acute liver injury and chronic liver disease may not be universally detrimental. The localization of hepatic fibrin(ogen) deposits within the liver is connected to the disease stimulus and in animal models of liver toxicity and chronic disease, fibrin(ogen) deposition may not always be synonymous with large vessel thrombosis. Here, we provide a balanced review of the experimental evidence supporting a direct connection between fibrin(ogen) and liver injury/disease pathogenesis, and suggest a path forward bridging experimental and clinical research to improve our knowledge on the nature and function of fibrin(ogen) in liver disease.

Keywords

Fibrin(ogen); liver; coagulation

The intersection between components of the hemostatic system and liver disease is complex and is the topic of ongoing clinical investigation and of basic research seeking mechanisms that link coagulation to the progression of liver disease. Strong experimental evidence supports the concept that coagulation protease activity, including that of thrombin, modifies the progression of acute liver injury and disease experimental settings as diverse as acetaminophen (APAP) hepatotoxicity and liver fibrosis.[1,2] Clinical studies in patients with

Address for correspondence: James P. Luyendyk, PhD, Department of Pathobiology & Diagnostic Investigation, Michigan State University, 1129 Farm Lane, 253 Food Safety and Toxicology Building, East Lansing, MI 48824, Phone: (517) 884-2057, luyendyk@cvm.msu.edu.
AKK: akopec@msu.edu

cirrhosis and experimental models of liver disease support the concept that coagulation in turn modifies hepatic disease pathogenesis.[1,3,4] Drawing from experimental genetic and pharmacologic interventions available in animal models has led to a rich literature identifying the mechanism(s) whereby coagulation contributes to the pathogenesis of liver injury/disease. Many studies have focused on thrombin proteolytic activity and its many substrates, including fibrin(ogen) and the protease activated receptors (PARs). There is vast literature linking the PARs to liver pathology, and this has been reviewed elsewhere.[1] In this review, we focus on fibrin(ogen), a prototypical substrate of thrombin often evaluated in experimental settings of liver injury/disease as an indicator of local (hepatic) thrombin activity. Our goal is to provide a balanced view on the proposed roles of fibrin(ogen) in experimental liver toxicity and disease, and to provide a concise review on studies that indirectly suggest or directly identify a role of fibrin(ogen) in liver disease. We do so by examining both acute and chronic liver damage models, primarily in rodents. Finally, we will briefly address the potential clinical implications of these results, particularly as related to potential utility of anticoagulants in patients with liver disease, along with underscoring the challenges and gaps in knowledge to be addressed by future studies.

Fibrin(ogen) consumption and hepatic deposition in acute liver injury

It was noted well over 100 years ago that the consumption of plasma fibrinogen occurred alongside induction of hepatic necrosis.[5] Since that time, multiple animal models have demonstrated a strong connection between experimental liver injury triggered by very diverse stimuli and the consumption of plasma fibrinogen. A non-exhaustive list of examples of this connection include models of liver injury caused by chloroform[5], carbon tetrachloride (CCl_4)[6], APAP[2,7], dimethylnitrosamine[8], alpha-naphthylisothiocyanate (ANIT)[9], Jo2 (Fas agonist, IgG)[10,11], endotoxin[12], ethanol[13], monocrotaline[14], ischemia-reperfusion (IR)[15], and bile duct ligation (BDL)[16,17]. Reflecting consumption of plasma fibrinogen induced by a hepatotoxic stimulus, fibrin(ogen) accumulation/deposition in the liver has been documented in several of these models, most often by immunohistochemical (IHC) staining. Of importance, the common utilization of polyclonal fibrin(ogen) antibodies for IHC makes it challenging to conclude that all positive stain represents fibrin polymer. Thus, these deposits are most often referred to as fibrin(*ogen*) deposits, denoting this possibility. Several studies note that administration of heparin or genetic deficiency in tissue factor attenuates both consumption of plasma fibrinogen and intrahepatic deposition of fibrin(ogen)[2,9,14,15], consistent with the hypothesis that thrombin-mediated consumption of fibrinogen drives fibrin(ogen) deposition in liver.

Although the exact nature of intrahepatic fibrin deposits is not well characterized, the localization of these fibrin(ogen) deposits by IHC is diverse and often reflective of the nature of the inciting cause of liver damage. For instance, administration of endotoxin (lipopolysaccharide, LPS) triggers intravascular thrombin generation via tissue factor expression and this is reflected in liver by a distinct deposition of fibrin(ogen) in the liver microvasculature (i.e., hepatic sinusoids) of exposed rats and mice.[12,18] Toxicants such as ethanol are capable of exaggerating this intravascular fibrin(ogen) deposition in experimental models of ethanol-potentiated LPS-induced liver injury.[19] Likewise, in hepatic IR-mediated liver injury, where the liver endothelium is damaged, sinusoidal fibrin(ogen)

deposits are readily observed.[15] Fibrin(ogen) deposition often localizes to injured areas of the liver, as is the case for liver damage triggered by toxicants damaging centrilobular hepatic parenchymal cells of the liver (i.e., zone 3). These include CCl_4, monocrotaline, and APAP, where very robust fibrin(ogen) deposition is observed, often directly demarcating the area of necrotic injury.[2,6,7,14] Acute cholestatic liver damage induced by either complete obstruction of the common bile duct or acute administration of a single large dose of ANIT to fasted mice produces yet another unique histological pattern of fibrin(ogen) deposition. Here, the primary lesion is characterized by accumulated components of bile and hepatocellular necrosis most often attributed to excessive neutrophil activation.[9,16,17] Fibrin(ogen) deposits are readily observed within these so-called “bile infarcts”. Hepatocyte apoptosis, such as that initiated with the Fas ligand-like antibody Jo2, is also associated with coagulation cascade activation and deposition of fibrin(ogen) in the liver, with the distinct pattern here being spider-like fibrils that appear to encase apoptotic hepatocytes.[11] Collectively, there is substantial diversity in the appearance and zonal distribution of fibrin(ogen) deposits in the acutely injured liver.

It seems that the location of fibrin(ogen) deposits closely relates to the region impacted by the injury, an observation that is perhaps not surprising given the close connection between tissue injury and coagulation. The diversity in appearance and location of fibrin(ogen) deposits after acute toxicity raises many unanswered questions as to the exact nature of these fibrin(ogen) deposits. Are they like traditional intravascular thrombi, and if so, do they resemble venous or arterial thrombosis? Alternatively, are these unique fibrin(ogen) deposits extravascular and driven by unique zonal disruption of the liver microvasculature? In a related question, it is not known whether the content of hepatic fibrin(ogen) deposits includes red blood cells or platelets. The presence of arterial and venous circulation within the liver along with sinusoids lined by unique fenestrated endothelium has a high potential to create a diversity in the structure and localization of fibrin(ogen) deposits in the diseased liver. Likewise, it is important to consider that plasma fibrinogen levels are dictated by a balance of consumption and synthesis, the latter being potentially altered in the diseased or injured liver.

Potential role of fibrin(ogen) in acute liver injury: Indirect and direct evidence

The reason for considering fibrin(ogen) deposits as a causal player in acute liver injury stems from numerous studies that have described a reduction in liver damage after administration of anticoagulant drugs. Examples of studies demonstrating a reduction in liver damage by administration of anticoagulant include APAP-induced hepatocellular necrosis, IR injury, LPS and LPS-potentiated hepatotoxic responses, and Jo2-induced liver apoptosis.[2,10,11,20-23] The reader is encouraged to note the diverse pattern of fibrin(ogen) deposition (above) despite commonality in the protection from injury afforded by anticoagulants. Although not always examined, it would be anticipated that anticoagulation would attenuate hepatic fibrin(ogen) deposition in each of these experimental settings. A commonly proposed mechanism whereby fibrin(ogen) deposits contribute to liver damage is akin to their role in thrombosis. That is, fibrin(ogen) could be deposited as intravascular

microthrombi, perhaps alongside platelets etc., that obstruct local hepatic blood flow causing secondary tissue injury. Although a leading hypothesis in the field, this has not been systematically investigated in many models.

Hepatic ischemia-reperfusion injury

Like other tissues, hepatic IR injury is associated with sinusoidal fibrin(ogen) deposition and platelet accumulation, leading to the potential for platelet-fibrin(ogen) interactions. The role of platelets in hepatic IR injury has been discussed previously.[24] Anticoagulation has been shown to reduce hepatic IR injury in multiple studies[15,25,26], suggesting the possibility that hepatic fibrin(ogen) deposits could contribute to hepatic IR injury. Several studies suggest that anticoagulation attenuates damaging hepatic inflammation, including hepatic chemokine induction and neutrophil accumulation/activation after hepatic IR. Also, interventions such as administration of the fibrin-derived Bβ15-42 peptide, a fibrin degradation product with anti-inflammatory properties, attenuates hepatic IR injury.[27] These studies implicate fibrin(ogen) deposits in the pathogenesis of hepatic IR injury. Taking cues from other IR injury models, complete fibrinogen deficiency was deleterious after kidney IR, whereas partial fibrinogen deficiency afforded moderate protection.[28] Infarct size was not affected by enzymatic depletion of fibrin(ogen) with ancrod in a model of cardiac IR.[29] However, studies examining the impact of selective fibrinogen deficiency on hepatic IR injury have not yet been published. Thus, while strong circumstantial evidence supports a pathologic role for fibrin(ogen) in hepatic IR injury, its precise role has not yet been defined.

LPS-induced liver injury in the rat

LPS-driven thrombin generation is tissue factor-dependent.[30] Although LPS requires a “potentiating” stimulus to provoke marked hepatotoxicity in mice, rats given a single dose of LPS alone develop marked midzonal, neutrophil-dependent liver injury.[31] Fibrin(ogen) deposits are evident in the liver sinusoids, as anticipated, and administration of a direct thrombin inhibitor reduces liver injury.[12] However, fibrin(ogen) depletion with ancrod afforded no protection form liver injury[12], suggesting that in this model thrombin-driven liver injury is independent of fibrin(ogen). Indeed, in an isolated-perfused liver system, PAR-1 activation appeared sufficient to drive neutrophil-mediated liver damage.[32]

Alcohol and LPS-potentiated hepatotoxicity

Activation of the coagulation cascade by endotoxin has been linked to the potentiation of liver injury induced by several xenobiotics, including several drugs.[19,23,33,34] Hepatic fibrin(ogen) deposits are frequently observed in the livers of rats/mice in these experimental settings, but there is very little experimental evidence to suggest this fibrin(ogen) deposition is pathologic. As an example, strong evidence indicates that thrombin inhibition reduces hepatic fibrin(ogen) deposition and liver injury caused by co-exposure to LPS and ethanol.[19] Furthermore, plasminogen activator inhibitor-1 (PAI-1) deficiency reduced liver pathology induced by ethanol alone and in combination with LPS.[19,35] These studies suggest that fibrin(ogen) deposition drives liver injury in these models, but experimental evidence is restricted to the effect of inhibiting thrombin deposition of fibrin(ogen) or modifying

fibrinolytic activity through PAI-1 deficiency. Similar studies done in rodent models combining LPS with drugs to induce hepatotoxicity have reported protection from injury by administration of thrombin inhibitor or PAI-1 deficiency.[34] Likewise, the role of fibrin(ogen) has not been specifically addressed.[34] Overall, the effect of fibrinogen deficiency has not been examined in this and other settings of LPS-potentiated hepatotoxicity.

Acetaminophen hepatotoxicity

As the leading cause of drug-induced liver failure, the mechanism of APAP hepatotoxicity has received considerable attention since its approval as an analgesic in the 1970s.[36] APAP overdose in mice recapitulates many primary features of APAP-induced liver injury in human patients and is widely utilized to decipher mechanisms of APAP hepatotoxicity. APAP overdose in mice is associated with tissue factor-mediated coagulation cascade activation, consumption of plasma fibrinogen, and hepatic fibrin(ogen) deposition.[2,37] Here, it is likely that injury to tissue factor-expressing hepatocytes drives coagulation cascade activation. Fibrin(ogen) deposition associates with areas of necrosis upon histological examination.[38] Administration of anticoagulants such as heparin or lepirudin significantly reduces hepatic fibrin(ogen) deposition as well as early APAP hepatotoxicity in mice[2,20], suggesting that hepatic fibrin(ogen) deposits may contribute to APAP-induced liver injury. However, enzymatic depletion of fibrin(ogen) with ancrod had no effect on APAP hepatotoxicity, despite markedly reducing hepatic fibrin(ogen) deposition.[38] Similarly, complete fibrinogen deficiency did not significantly affect APAP-induced liver injury, assessed by increased serum alanine aminotransferase (ALT) activity.[38] These studies suggest that fibrin(ogen) is not essential for APAP hepatotoxicity in mice. Rather, thrombin activation of PAR-1 and PAR-4 appears central to exacerbation of early APAP-induced liver injury in mice.[2,20] Furthermore, there appears to be a distinct role for plasminogen activation in APAP hepatotoxicity. PAI-1-deficient mice develop significantly worse liver damage after APAP overdose, whereas mice completely lacking plasminogen display a reduction in early liver injury.[38,39]

Fas-induced liver apoptosis

Activation of Fas expressed by hepatocytes increases tissue factor procoagulant activity and Fas-induced hepatocellular apoptosis *in vivo* is driven by liver tissue factor.[11] The accumulation of fibrin(ogen) γ chain and its dimers in liver after administration of Jo2 (a FasL-like IgG) has been elegantly described.[10] In these studies, heparin administration significantly reduced secondary necrosis and delayed lethality without impacting induction of apoptosis.[10] Another study found that liver tissue factor deficiency reduced secondary necrosis after Jo2 administration, and noted that intrahepatic fibrin(ogen) deposits surrounded apoptotic hepatocytes.[11] Collectively, these studies are consistent with a role for coagulation in promoting liver injury. However, to date there is no published evidence definitively identifying a pathologic role for fibrin(ogen) in this model of liver injury.

Acute cholestatic liver injury

Acute cholestatic liver injury is effectively modeled by ligating the common bile duct or administration of a large, hepatotoxic dose of ANIT to fasted mice/rats. Hepatocellular injury occurs rapidly after common BDL and is typified by acute focal necrosis driven by neutrophil activation, platelets and bile acid-mediated inflammation.[40,41] Administration of ANIT by oral gavage produces near identical lesions, albeit at a later time point (~24-48 hours) owing to the time required for ANIT to achieve toxic levels in the bile.[42] Like BDL, necrosis after acute ANIT administration is mediated by neutrophils and platelets.[43-45] Both ANIT- and BDL-mediated liver injury are associated with coagulation activation and intrahepatic deposition of fibrin(ogen).[9,46] In each model, the latter is driven by tissue factor[9,47] and is most evident within areas of necrosis. Interestingly, however, tissue factor deficiency reduces necrosis after acute ANIT administration, but has no effect on liver injury after BDL.[9,47] Worth noting, the latter BDL study examined extended time points associated with fibrosis and not early hepatocellular injury. Other studies certainly suggest a role for coagulation in early BDL-induced liver injury.[48] Notably, PAI-1 deficiency reduces liver injury after BDL[16,17] and tissue plasminogen activator (tPA) deficiency worsens BDL-induced liver injury.[49] These changes are reflected by the anticipated changes in hepatic fibrin(ogen) deposition (i.e., decreased in PAI-1-deficient mice and increased in tPA-deficient mice), suggesting a potential pathologic role of fibrin(ogen) in liver injury after BDL. However, the role of fibrin(ogen) after BDL has not yet been directly tested. In unpublished studies we found that complete fibrinogen deficiency tended to increase serum ALT activity 48 hours after BDL (*unpublished results*, Luyendyk and Copple). Additional studies are required to delineate the exact role of fibrin(ogen) in the setting of experimental BDL. Interestingly, complete fibrinogen deficiency reduced acute ANIT-induced liver necrosis, whereas PAR-1 deficiency had no impact on injury[50], suggesting that in the context of acute xenobiotic-mediated cholestatic liver necrosis fibrin(ogen) plays a pathologic role. Notably, when ANIT-induced bile duct injury is allowed to persist, fibrin(ogen) takes on a protective function, highlighting how the duration and intensity of disease stimulus can dictate the particular role of fibrin(ogen). This will be discussed in the chronic fibrosis section.

Acute liver injury: Summary

Increased fibrin(ogen) deposition is a common feature of acute liver injury caused by very diverse stimuli. Its zonal distribution appears to be driven by the nature of the injurious stimuli (Supplemental Figure 1) and for very few studies has its role been entirely defined. Where the role of fibrin(ogen) has been directly tested, available evidence suggests it is not essential for liver damage. However, it remains very plausible that fibrin(ogen) could play a central role in some types of liver injury, particularly given the potential for sinusoidal occlusion or inflammatory cell engagement/recruitment. However, for the vast majority of models this remains a very exciting, yet untested hypothesis. There is ample opportunity for clinical evaluation of concepts revealed by studies examining coagulation in acute liver damage. Clear evidence indicates a hypercoagulable state in patients with acute liver failure, most often caused by acetaminophen overdose.[51] Moreover, there is evidence that post-liver transplantation[52] and after surgical liver resection[53] are associated with features of a

hypercoagulable state. Defining the precise role of coagulation, and particularly fibrinogen function, in these clinical settings may have significant value in terms of improving outcome in patients.

Fibrin(ogen) in chronic liver disease and fibrosis

Several studies highlight complex changes in the hemostatic system in patients with various chronic liver diseases. Patients with severe liver disease, particularly cirrhosis, have a rebalanced hemostatic system, and are prone to both bleeding and thrombosis.[54] There is an obvious interest in building knowledge in how fibrinogen expression and plasma levels are affected by liver disease, given its central role in both hemostasis and thrombosis. In addition, there is an emerging and important interest in how fibrin structure is impacted by concurrent liver disease.[55] As a central element of venous clots[56], fibrin(ogen) is likely to contribute to pathologies associated with thrombotic complications of chronic liver disease, such as portal vein thrombosis. Less clear at this time is whether fibrinogen or intrahepatic fibrin(ogen) deposits, perhaps through mechanisms traditionally linked to thrombosis, could contribute to the progression of liver disease. There are many distinct models of chronic liver disease in rodents that recapitulate several aspects of the pathologies associated with human liver disease. By definition, these models cannot fully copy the human condition, but nonetheless they are exceedingly applicable as experimental settings to ask mechanistic questions on the role of hemostatic system components in chronic liver disease and fibrosis. Here, we will review some of the commonly utilized models of chronic liver disease in rodents and delineate what is known on the role of fibrin(ogen) in liver and systemic pathologies associated with these experimental approaches.

Liver fibrosis

Liver fibrosis is the penultimate outcome of multiple chronic liver diseases wherein extracellular matrix, particularly collagens, is produced in excess and over time replaces the functional liver parenchyma. The cell types widely considered to be responsible for producing excess collagen are hepatic stellate cells and also to some degree, portal fibroblasts.[57] Severe liver fibrosis, termed cirrhosis, is a common indication for liver transplantation. Although difficult to treat, cirrhosis is increasingly regarded as treatable and anticoagulants are among classes of drugs considered as potential therapies for liver fibrosis.[58,59] Experimental approaches to induce liver fibrosis in rodents are diverse, but share a common theme in that the presence of fibrosis is underpinned by chronic liver injury. The cellular mechanism driving liver injury in each setting varies, as does the distribution of collagen deposits throughout the liver. However, for many of these models the pattern of injury and fibrosis resembles that observed in humans with certain liver diseases, and the mechanism whereby coagulation protease activity drives disease has received detailed attention.

Carbon tetrachloride

CCl_4 is a well-studied hepatotoxic carcinogen. CCl_4 is metabolized by cytochrome P450 enzymes in the liver to a free radical that triggers oxidative stress and hepatotoxicity.[60] CCl_4

hepatotoxicity is characterized by acute centrilobular necrosis, and as noted earlier, acute CCl_4 administration is associated with consumption of plasma fibrin(ogen) and deposition of fibrin(ogen) within the injured liver.[61,62] Some studies suggest a deleterious contribution of the hemostatic system in acute CCl_4 hepatotoxicity, whereas others have found that anticoagulant administration is without effect.[6,8,48,63,64] CCl_4 hepatotoxicity represents one of the first models utilized to carefully evaluate changes in fibrin(ogen) after the induction of acute liver damage.[61] Interestingly, and like APAP overdose, complete fibrinogen deficiency did not affect acute CCl_4 hepatotoxicity or injury resolution in mice.[65] Interestingly, factor XIII deficiency delayed hepatocyte proliferation after acute CCl_4 administration, suggesting a potential role of fibrin(ogen) cross-linking in liver repair.[66]

Administration of CCl_4 twice weekly for several weeks (typically at least 4-8 weeks) results in hepatic fibrosis in mice and rats. For these studies the primary endpoint is measurement of excess hepatic collagen deposition. A contribution for the hemostatic system components, including fibrin(ogen), in this process has long been hypothesized. Indeed, strong evidence supports a role for the hemostatic system in hepatic fibrosis induced by chronic CCl_4 administration.[67] Administration of anticoagulants reduces hepatic fibrosis whereas mice harboring the procoagulant factor V Leiden mutation develop increased fibrosis.[68,69] Fibrin(ogen) deposition in livers of mice exposed chronically to CCl_4 parallels collagen deposits in terms of localization.[70] In elegant studies seeking to understand the role of plasminogen in liver repair after chronic CCl_4 administration, complete fibrinogen deficiency was observed to have no effect on gross liver pathology, and did not rescue the defect in liver repair associated with plasmin(ogen) deficiency.[71] Thus, it appears as though fibrin(ogen) does not play a major role in liver fibrosis induced by chronic exposure to CCl_4. Suggesting an alternate path whereby the hemostatic system drives fibrosis after chronic CCl_4 administration, mice deficient in either PAR-1 or PAR-2 have reduced fibrosis in this model.[72,73] This implies that thrombin and/or factor Xa signaling drive liver fibrosis either directly through stellate cell activation[74] or indirectly via leukocyte activation[75].

Cholestasis-associated fibrosis

Bile duct injury is a feature of liver diseases such as primary sclerosing cholangitis and primary biliary cirrhosis, and a consequence of the obstruction of bile flow in the common bile duct. Obstructive cholestasis is modeled in the mouse by BDL, which we discuss in the *acute liver injury* section of this review. If allowed to persist, liver fibrosis develops. As noted earlier, the precise role for fibrin(ogen) in this model has not been studied, although tissue factor deficiency did not impact fibrosis in this model.[47] Thrombocytopenia significantly reduces hepatocellular necrosis after BDL[76,77], but in an interesting dichotomy, this also increases liver fibrosis after BDL.[77] Of importance, the connection of this observation to fibrin(ogen) function is not known. Autoimmune-mediated primary biliary cirrhosis is modeled by multiple strategies, including genetic modification in mice[78], although the role of hemostatic components in these models has not been definitively addressed. Among the models of primary sclerosing cholangitis is the $Mdr2^{-/-}$ mouse, which lacks a phospholipid transporter essential for maintaining appropriate bile flow.[79] Although there is some suggestion that platelets could contribute to liver disease in $Mdr2^{-/-}$

mice[80], the precise role of other hemostatic system components, including thrombin and fibrin(ogen), has not yet been evaluated in Mdr2$^{-/-}$ mice.

Our laboratory and others have capitalized on the depth of toxicological insight in the ANIT model (described previously in *acute liver injury* section) to refine a model of xenobiotic-induced bile duct hyperplasia and peribiliary fibrosis. In this model, mice are exposed to low levels (typically up to 0.1%) of ANIT formulated into chow (i.e., ANIT diet).[81-83] Whereas a single acute dose of ANIT by gavage elicits marked hepatotoxicity, hepatocellular necrosis is relatively infrequent in mice exposed to ANIT diet.[9,50] Rather, bile duct hyperplasia, lymphocytic infiltration, and peribiliary fibrosis are the primary lesions.[9,84,85] Evidence supporting coagulation cascade activation in mice exposed to ANIT diet includes increased plasma thrombin-antithrombin levels and deposition of fibrin(ogen) within areas of hepatocellular necrosis, sinusoids and surrounding bile ducts.[82,84] Similar to CCl_4-induced liver fibrosis, it appears that coagulation drives hepatic fibrosis, as both tissue factor and PAR-1 deficiency each reduced hepatic collagen deposition in mice exposed to ANIT diet.[84]

Interestingly, complete fibrinogen deficiency increased liver injury in mice exposed to ANIT diet[81], a result quite the opposite from the protection conferred by complete fibrinogen deficiency in mice given a single large hepatotoxic dose of ANIT.[50] This difference highlights how the exact role of fibrin(ogen) may vary depending on the duration, intensity, and nature of liver injury. In the context of ANIT diet, PAI-1 deficiency increased liver injury and fibrosis, consistent with a protective effect conferred by PAI-1.[86] Also, administration of the antifibrinolytic drug tranexamic acid reduced liver fibrosis in mice exposed to ANIT diet.[86] The mechanism whereby fibrin(ogen) appears to inhibit hepatocellular necrosis in ANIT diet exposed mice appears to relate to the engagement of platelets via the $\alpha_{IIb}\beta_3$ integrin. ANIT diet-induced hepatocellular injury and fibrosis are significantly increased in Fib$\gamma^{\Delta 5}$ mice, which express a mutant fibrinogen that lacks the binding domain for $\alpha_{IIb}\beta_3$.[82] Collectively, these studies suggest that in some experimental settings fibrin(ogen) can reduce some facets of experimental liver disease. The majority of these fibrin(ogen)-directed interventions has not been examined in other models of cholestatic liver injury and fibrosis, and much more experimentation is required to determine whether there is consensus across the various models.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome and obesity and is a major contributor to liver-associated morbidity. Although further research is required, some studies have suggested that prothrombotic risk factors are associated with the progression of this spectrum disorder to its more severe form, non-alcoholic steatohepatitis (NASH).[87,88] Among changes considered is the hepatic expression of fibrinogen, which is likely to reflect hepatic inflammation along with other acute phase proteins.[89] It is very difficult to decipher whether these changes occur simply as a function of worsening liver disease, or whether the coagulation drives liver disease. To our knowledge there is not yet a definitive study demonstrating beneficial effects of anticoagulants in patients with NAFLD/NASH. However, a number of studies have suggested a role for the hemostatic system in the progression of experimental NAFLD/NASH. Procoagulant and

signaling functions of tissue factor have been shown to drive various features of NAFLD including hepatic steatosis and inflammation.[90-92] Experimental inhibition of thrombin activity significantly attenuates body weight gain and obesity-associated pathologies, including NAFLD/NASH.[93,94]

Fibrin(ogen) deposition is evident in livers of mice fed a methionine-choline deficient diet and in mice fed a high fat diet.[90,91,93] Fibrin(ogen) deposits in this case are primarily sinusoidal. To date, no published study has definitively identified a role for fibrin(ogen) in experimental obesity-associated liver disease. It is clear that experimental NAFLD/NASH is driven in part by PAR-1.[90] However, PAR-1 deficiency does not explain the reduction in body weight gain observed in tissue factor-deficient mice and mice given a direct thrombin inhibitor. Thus, it is likely that another thrombin target, potentially fibrin(ogen), plays a central role in experimental obesity. Fibrin(ogen) could exacerbate hepatic inflammation associated with NAFLD/NASH. It is possible that sinusoidal occlusion by fibrin(ogen) deposits could cause hypoxia, which is well known to exacerbate NAFLD/NASH pathology. Moreover, it is entirely possible that the deposition of fibrin(ogen) in other tissues, such as adipose, contributes to local and systemic changes that ultimately drive NAFLD as a component of obesity. Although intriguing hypotheses, further studies are needed both in experimental settings and in human patients to determine the precise cause-effect relationship between the hemostatic system and obesity-associated pathologies.

Summary, gaps in knowledge, and clinical implications

Although diverse in appearance and localization, intrahepatic fibrin(ogen) deposition is a very common observation in models of acute and chronic liver disease. Although many studies have observed a role for the hemostatic system in experimental liver disease, most studies have not directly evaluated the role of fibrin(ogen) (Table 1). Where fibrin(ogen) function in liver disease has been examined, in many cases it is not essential for hepatotoxicity, as in the case of CCl_4, APAP, and LPS (rat) hepatotoxicity. In other models, such as ANIT, the role of fibrin(ogen) depends on the nature of the stimulus and the liver pathology produced. Overall, there is much more to be learned on the precise function of fibrin(ogen) in acute and chronic liver injury.

Experimental needs include a more in-depth characterization of plasma and hepatic fibrin(ogen) expression in models of chronic liver disease. As an acute phase protein, fibrinogen plasma levels are likely to vary with hepatic inflammation, and may differ between diseases. Although there is a suggestion of this in patients[95], there is a paucity of studies that have examined this experimentally. A large gap in our knowledge relates to the exact disposition of fibrin(ogen) in liver. There is a need to improve upon detection methods, which by-in-large rely on approaches incapable of distinguishing fibrin polymer from deposited soluble fibrinogen. It is also unclear whether intrahepatic fibrin(ogen) deposits resemble arterial or venous thrombi, or are a completely unique entity. Unlike large vessel thrombi, which can more easily be surgically collected for analysis, selective isolation of fibrin(ogen) deposits from the liver is likely very difficult. Application of such an approach in combination with detection of cross-linked fibrin polymer would prove very valuable. Finally, increased utilization of mice with fibrinogen deficiency, or expressing fibrinogen

with modified function(s) is likely to yield very robust mechanistic insight into the exact role of fibrin(ogen) *in vivo.*

In considering the clinical implications of experimental findings related to fibrin(ogen) in liver disease, it will be important to define whether fibrin(ogen) deposits are present in the diseased human liver. This has not been examined systematically, and is beset by the same challenges facing basic scientists, as well as the variable of *ex vivo* coagulation occurring during sample collection. It remains plausible, however, that intrahepatic fibrin(ogen) deposition, perhaps distinct from more traditional thrombosis, is present in patients with liver disease. Utilizing an anti-fibrin monoclonal antibody, we identified hepatic fibrin deposits in snap-frozen liver samples removed from cirrhotic patients that had primary sclerosing cholangitis.[81] Although suggestive, results of this type are just the tip of the iceberg and much more study is needed. The abundance of studies indicating stasis drives complications such as thrombosis in cirrhotic patients makes new models such as inferior vena cava (IVC) ligation-driven coagulation and liver fibrosis very exciting[96], although it will be important to define whether anticoagulants have a beneficial effect by reducing thrombosis at the site of IVC stenosis or by reducing intrahepatic fibrin(ogen) deposition. Several published and ongoing clinical studies have sought to define the impact of anticoagulation in liver disease patients. This has been discussed recently.[3] Although a primary goal of these studies was to treat or prevent portal vein thrombosis, there may be a reduction in liver disease-driven fibrin(ogen) deposition. Whether the benefits of anticoagulant therapy are directly connected to fibrin(ogen) is an exceedingly difficult question to address in patients. This is certainly a logical conclusion, as a reduction in thrombosis is likely to have a positive impact on liver function. However, anticoagulants may also block PAR-1 signaling, which experimental studies suggest drives liver fibrosis. It is also possible that fibrin(ogen) deposits have beneficial function, such as a role in tissue repair.

Based on experimental evidence, it may not be appropriate to universally assume all fibrin(ogen) deposits in liver are pathologic. The role of fibrin(ogen) in liver disease may depend on variables like the location and structural/biochemical nature of the fibrin(ogen) deposit as well as the disease itself. With respect to fibrin(ogen) and liver disease, there is a true opportunity for creative translation of basic research to the clinical setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Role of hemostatic system and fibrinogen in models of liver disease

Liver disease model	Activation of coagulation?[a]	Effects of hemostatic system modification on the liver disease?	Role of fibrinogen?	References
Hepatic ischemia/reperfusion	√	Anticoagulation: ↓ liver injury	Unknown	15, 25, 26
Lipopolysaccharide (LPS) administration	√	Thrombin inhibition: ↓ liver injury	Fibrinogen deficiency (ancrod): no effect on liver injury	12, 31
Ethanol/LPS co-exposure	√	Thrombin inhibition: ↓ liver injury PAI-1 deficiency: ↓ liver injury	Unknown	19,35
LPS-potentiated hepatotoxic responses	√	Thrombin inhibition: ↓ liver injury PAI-1 deficiency: ↓ liver injury	Unknown	23, 33, 34
Acetaminophen toxicity	√	Thrombin inhibition: ↓ liver injury PAR-1 deficiency: ↓ liver injury PAR-4 deficiency: ↓ liver injury PAI-1 deficiency: ↑ liver injury Plasminogen deficiency: ↓ liver injury	Fibrinogen deficiency (ancrod): no effect on liver injury Fibrinogen deficiency ($Fga^{-/-}$): no effect on liver ALT levels	2, 20, 38, 39
Jo2-induced apoptosis	√	Thrombin inhibition: ↓ secondary liver necrosis Tissue factor deficiency: ↓ secondary liver necrosis	Unknown	10, 11
Acute cholestasis by alpha-naphthylisothiocyanate (ANIT)	√	Tissue factor deficiency: ↓ liver injury PAR-1 deficiency: no effect on liver injury	Fibrinogen deficiency ($Fga^{-/-}$): ↓ liver injury	9, 50
Obstructive cholestasis by bile duct ligation (BDL)	√	PAI-1 deficiency: ↓ liver injury tPA deficiency: ↑ liver injury	Unknown	16, 17, 49
Acute carbon tetrachloride (CCl4) toxicity	√	Anticoagulation: ↑↓liver injury	Fibrinogen deficiency ($Fga^{-/-}$): no effect on liver injury	8, 48, 64, 65
Chronic CCl4 toxicity	√	Anticoagulation:↓ liver fibrosis Factor V Leiden mutation: ↑ liver fibrosis PAR-1 deficiency: ↓ liver fibrosis PAR-2 deficiency:↓ liver fibrosis	Fibrinogen deficiency ($Fga^{-/-}$): no effect on gross liver pathology	68, 69, 71, 72, 73
Cholestasis-associated fibrosis after BDL	√	Tissue factor deficiency: no effect on liver fibrosis Thrombocytopenia: ↓ liver necrosis Thrombocytopenia: ↑ liver fibrosis	Unknown	47, 76, 77
Cholestasis-associated fibrosis after chronic ANIT feeding	√	Tissue factor deficiency: ↓ liver fibrosis PAR-1 deficiency: ↓ liver fibrosis	Fibrinogen deficiency ($Fga^{-/-}$): ↑liver injury	81, 82, 84, 86

Liver disease model	Activation of coagulation?[a]	Effects of hemostatic system modification on the liver disease?	Role of fibrinogen?	References
		PAI-1 deficiency: ↑liver injury and liver fibrosis		
		Tissue factor/PAR-2 signaling: ↑ liver injury, steatosis, obesity Tissue factor deficiency: ↓liver injury, steatosis		
Non-alcoholic fatty liver disease	√	Thrombin inhibition: ↓ liver injury, steatosis, obesity PAR-1 deficiency: ↓liver injury, steatosis	Unknown	90, 91, 92, 93, 94

[a] Activation of coagulation defined as evidence of thrombin generation, depletion of plasma fibrinogen, and/or thrombin-mediated hepatic fibrin(ogen) deposition