Published in final edited form as:
Thromb Res. 2014 May ; 133(0 1): S57–S59. doi:10.1016/j.thromres.2014.03.023.

Coagulation in liver toxicity and disease: Role of hepatocyte tissue factor

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

[1]Department of Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, MI 48824

Abstract

The liver is the primary source of a number of circulating coagulation factors, and acute liver injury and chronic liver disease are each associated with alterations in blood coagulation. Current views of the connection between liver injury and coagulation extend beyond the impact of liver disease on synthesis of coagulation factors to include a role for coagulation factor activity in the initiation and progression of liver disease. Mechanisms of coagulation initiation in liver disease are not completely understood. Compared to other tissues, liver expresses very low levels of tissue factor (TF). Recent studies indicate that expression of TF by hepatocytes comprises the majority of liver procoagulant activity, and that hepatocyte TF activates coagulation induced by liver injury. This review will briefly cover the expression and regulation of TF by hepatocytes, the role of TF in coagulation triggered by liver toxicity, and the contribution of coagulation activity to the progression of liver disease.

Perturbations in the coagulation cascade are frequently associated with acute liver toxicity and chronic liver disease [1]. In few cases is the etiology of these disturbances understood in great detail. The liver is the primary site of synthesis of nearly all coagulation factors, along with several proteins involved in fibrinolysis and anticoagulation. Chronic liver disease or acute hepatotoxicity can significantly alter the hepatic synthesis of these factors, and thus the systemic balance and overall levels of pro-and anticoagulant factors [1]. This is increasingly recognized as a component of liver disease pathogenesis [2]. The consequence of acute toxic liver injury can include consumptive coagulopathy, whereas in patients with chronic liver disease and/or cirrhosis, a rebalanced, but unstable hemostatic state can easily shift to either hypo-or hypercoagulability [1].

[*]**Corresponding author**: James P. Luyendyk, Ph.D. Pathobiology & Diagnostic Investigation, Michigan State University, 348 Food Safety and Toxicology Building, 1129 Farm Lane, East Lansing, MI 48824, 517-884-2057, 517-432-2310 (fax) luyendyk@cvm.msu.edu.

Conflict of Interest Statement:
None relevant to this article.

Strong evidence supports a reciprocal relationship between liver disease and coagulation, whereby altered liver function results in coagulation anomalies, and also where coagulation protease activity contributes to the pathogenesis of liver disease. The coagulation protease thrombin and protease activated receptors (PARs) have been implicated in various facets of liver pathology in rodents [3]. Moreover, recent studies suggest that coagulation is associated with morbidity and mortality in patients with chronic liver disease [4, 5]. Therefore, understanding coagulation cascade perturbations in the context of acute and chronic liver disease is important. Emerging evidence suggests that tissue factor (TF), the primary activator of the blood coagulation cascade, is a critical mediator of coagulation in liver disease. Our goals here are to briefly review 1) the role of TF in coagulation cascade activation in models of liver disease, 2) evidence from mouse models implicating coagulation in liver pathology, and 3) highlight recent findings and unanswered questions related to the expression of TF by liver parenchymal cells (hepatocytes).

TF is the transmembrane receptor for coagulation factor VII/VIIa and the primary activator of the blood coagulation cascade. TF plays a central role in the hemostatic response to vessel injury [6] and is expressed at high levels to limit bleeding in critical organs/tissues including brain, heart, lungs, kidney, and placenta [7, 8]. At the cellular level, restricting expression of TF to extravascular cells prevents inappropriate clotting, but also keeps TF in a position juxtaposed to blood in order to rapidly activate coagulation secondary to loss of vascular integrity.

The liver expresses very low levels of TF mRNA and the livers of low TF mice appear normal [9]. The physiological reason for low expression of TF in the liver is not completely understood, but may relate to the architecture and function of the unique capillaries, termed liver sinusoids. Arterial and portal venous blood mix and percolate through the liver sinusoids, which are home to at least 15 different cell types, including hepatic stellate cells [10], immune cells including resident liver macrophages (i.e., Kupffer cells) [11], and sinusoidal endothelial cells [12]. In contrast to vascular endothelium, sinusoidal endothelial cells (SECs) are fenestrated, allowing for near free exchange of plasma components with hepatocytes lying just opposite the SECs across the space of Disse [12]. Hepatocytes occupy approximately 60% of the total liver cell population and carry out a number of critical liver functions, including coagulation factor synthesis. The “leaky” environment in the liver sinusoids poses a substantial regulatory challenge for the hemostatic system. Several studies suggest that disruption of anticoagulant factors results in hepatic fibrin deposition [13, 14], implying a microenvironment sensitive to subtle changes in the balance between pro-and anticoagulant factors.

Albeit at much lower levels, procoagulant TF is expressed in the liver. The procoagulant activity of mouse liver homogenate is reduced by >95% in livers from low TF mice [15], which express human TF at approximately 1% of normal TF levels [9]. An increasing number of studies indicate that TF-dependent coagulation accompanies hepatotoxic responses. For example, acetaminophen (APAP) overdose, the number one cause of drug-induced liver injury in the United States [16], is associated with coagulation cascade activation in humans [17] and increased TF-positive microparticles are associated with mortality in APAP overdose patients [4]. APAP hepatotoxicity in mice is associated with a

large increase in plasma TAT levels, which is entirely TF-dependent [18]. APAP-induced hepatotoxicity is reduced in low TF mice and also in PAR-1-deficient mice [18]. In contrast, fibrinogen deficiency does not affect early APAP hepatotoxicity [19]. These studies suggest that coagulation amplifies APAP hepatotoxicity, in part through PAR-1 signaling.

Nearly 25 years ago the capacity of hepatocyte membranes to convert coagulation factor X to Xa was reported, and this activity was speculated to be that of TF [20]. Since that time, additional studies demonstrated that isolated human hepatocytes have TF activity [21, 22]. To determine the contribution of hepatocyte TF to liver procoagulant activity, we crossed $TF^{flox/flox}$ mice with Albumin-Cre mice ($TF^{flox/flox}$/AlbCre mice), in order to reduce TF expression in hepatocytes. This approach proved highly efficient at deleting TF expression in mouse hepatocytes, and isolated hepatocyte procoagulant activity was entirely dependent on TF [23]. Total liver procoagulant activity was reduced by approximately 80% in livers of $TF^{flox/flox}$/AlbCre mice [23] and coagulation activation in mice after APAP overdose was nearly prevented in $TF^{flox/flox}$/AlbCre mice [23]. This suggests hepatocyte TF contributes to a procoagulant response triggered by hepatotoxicant exposure, although it is worth noting that emerging evidence suggests that albumin may also be expressed by hepatic stellate cells [24]. This has important implications for interpretation of results from $TF^{flox/flox}$/AlbCre mice.

The expression of TF by hepatocytes presents a serious regulatory challenge for the liver sinusoids because 1) owing to a leaky endothelium, hepatocytes are persistently bathed in plasma, and 2) hepatocytes are the primary cellular source of FVII/FVIIa. Our laboratory has adopted the analogy of hepatocyte TF as a “match in a dynamite factory.” The existence of TF in this premiere environment for coagulation without evidence of pathologic coagulation is striking, and likely forms a basis in the concept of TF encryption. Encrypted TF lacks procoagulant activity, but can be complexed with FVII/FVIIa [25]. Indeed, we found that >90% (often 100%) of TF procoagulant activity on isolated mouse and human hepatocytes was encrypted ([23] and unpublished results). TF-dependent generation of FXa by isolated hepatocytes did not require exogenous FVIIa, implying the TF molecule on these cells is “preloaded” with FVII/FVIIa [23]. Such a scenario is not unique to the liver, with encrypted TF:FVIIa complex also present in other vascular beds [26]. Hepatocytes may offer a novel opportunity to study mechanisms of TF encryption, although it is conceivable that the isolation process itself alters TF activity. Thus, for these studies, pairing primary isolated cells with other hepatocyte-like cells, such as HepaRG cells, could be of value.

Defining the mechanisms whereby hepatocyte TF procoagulant activity is encrypted will substantially increase our knowledge on the regulation of hemostasis in liver, and the basis for pathologic coagulation accompanying acute and chronic liver disease. Established mechanisms of TF decryption have been reviewed recently [25]. Disruption of phospholipid asymmetry, favoring increased phosphatidylserine on the cell surface, an important molecular gatekeeper for TF procoagulant activity, or rearrangement of extracellular disulfides [25], are each possible mechanisms controlling hepatocyte TF activity. Worth noting, hepatocytes are known to express protein disulfide isomerase on the extracellular membrane, which is postulated to participate in isomerization of a disulfide bond (Cys186-Cys208) on the TF molecule that controls procoagulant and signaling activity of TF [25]. TF

may also be expressed on the hepatocyte cannalicular membrane, a region rich in transporters that contribute to bile formation, but not exposed to blood [27]. Our laboratory is currently exploring these and other possibilities with the intent of defining the mechanism of TF encryption on hepatocytes.

Liver injury and dysfunction have emerged as features of chronic diseases associated with altered hemostatic activity (e.g., obesity), making it possible that hepatocyte TF contributes to other diseases in addition to acute toxicity. Coagulation activity is now viewed as a contributor to the pathogenesis of liver toxicity and disease, with TF identified in model systems as critical (Table 1). The reader is encouraged to seek recent reviews on the topic. Indeed, players in the hemostatic system including thrombin, and PAR-1 have each been shown to play a role in pathologies ranging from acute hepatotoxicity to steatosis and liver fibrosis [15, 18, 28, 29]. It is noteworthy that a recent clinical trial demonstrated an unanticipated reduction in hepatic decompensation in patients treated with low molecular weight heparin [5]. Hepatocyte TF also likely participates in the procoagulant response associated with transplantation of these cells for the treatment of liver disease [23, 30]. Additional translational studies are required to broaden the connection between these basic and clinical observations.

In summary, compared to other tissues, the liver expresses lower levels of TF and the majority of liver TF is expressed by hepatocytes. Nonetheless, recent studies have demonstrated that this source of TF serves an essential role in blood coagulation, particularly under pathologic conditions. Additional studies of the regulation and activation of hepatocyte TF are likely to identify novel mechanisms of coagulation activation in various liver diseases.

Acknowledgments

This work was supported by grants R01 ES017537 and R01 DK087886 from the National Institutes of Health.

References

1. Lisman T, Caldwell SH, Burroughs AK, Northup PG, Senzolo M, Stravitz RT, et al. Hemostasis and thrombosis in patients with liver disease: the ups and downs. Journal of hepatology. 2010; 53:362–371. [PubMed: 20546962]
2. Northup PG, Sundaram V, Fallon MB, Reddy KR, Balogun RA, Sanyal AJ, et al. Hypercoagulation and thrombophilia in liver disease. Journal of thrombosis and haemostasis : JTH. 2008; 6:2–9. [PubMed: 17892532]
3. Anstee QM, Dhar A, Thursz MR. The role of hypercoagulability in liver fibrogenesis. Clinics and research in hepatology and gastroenterology. 2011; 35:526–533. [PubMed: 21570930]
4. Stravitz RT, Bowling R, Bradford RL, Key NS, Glover S, Thacker LR, et al. Role of procoagulant microparticles in mediating complications and outcome of acute liver injury/acute liver failure. Hepatology. 2013; 58:304–313. [PubMed: 23389887]
5. Villa E, Camma C, Marietta M, Luongo M, Critelli R, Colopi S, et al. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. Gastroenterology. 2012; 143:1253–1260. e1-4. [PubMed: 22819864]
6. Tilley R, Mackman N. Tissue factor in hemostasis and thrombosis. Seminars in thrombosis and hemostasis. 2006; 32:5–10. [PubMed: 16479457]
7. Mackman N. Tissue-specific hemostasis in mice. Arteriosclerosis, thrombosis, and vascular biology. 2005; 25:2273–2281.

8. Pawlinski R, Fernandes A, Kehrle B, Pedersen B, Parry G, Erlich J, et al. Tissue factor deficiency causes cardiac fibrosis and left ventricular dysfunction. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:15333–15338. [PubMed: 12426405]
9. Parry GC, Erlich JH, Carmeliet P, Luther T, Mackman N. Low levels of tissue factor are compatible with development and hemostasis in mice. The Journal of clinical investigation. 1998; 101:560–569. [PubMed: 9449688]
10. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiological reviews. 2008; 88:125–172. [PubMed: 18195085]
11. Roberts RA, Ganey PE, Ju C, Kamendulis LM, Rusyn I, Klaunig JE. Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis. Toxicological sciences : an official journal of the Society of Toxicology. 2007; 96:2–15. [PubMed: 17122412]
12. Braet F, Wisse E. Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: a review. Comparative hepatology. 2002; 1:1. [PubMed: 12437787]
13. Maroney SA, Cooley BC, Sood R, Weiler H, Mast AE. Combined tissue factor pathway inhibitor and thrombomodulin deficiency produces an augmented hypercoagulable state with tissue-specific fibrin deposition. Journal of thrombosis and haemostasis : JTH. 2008; 6:111–117. [PubMed: 17973652]
14. Safdar H, Cheung KL, Salvatori D, Versteeg HH, Laghmaniel H, Wagenaar GT, et al. Acute and severe coagulopathy in adult mice following silencing of hepatic antithrombin and protein C production. Blood. 2013; 121:4413–4416. [PubMed: 23550037]
15. Luyendyk JP, Cantor GH, Kirchhofer D, Mackman N, Copple BL, Wang R. Tissue factor-dependent coagulation contributes to alpha-naphthylisothiocyanate-induced cholestatic liver injury in mice. American journal of physiology Gastrointestinal and liver physiology. 2009; 296:G840–G849. [PubMed: 19179621]
16. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, et al. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology. 2005; 42:1364–1372. [PubMed: 16317692]
17. Kerr R, Newsome P, Germain L, Thomson E, Dawson P, Stirling D, et al. Effects of acute liver injury on blood coagulation. Journal of thrombosis and haemostasis : JTH. 2003; 1:754–759. [PubMed: 12871412]
18. Ganey PE, Luyendyk JP, Newport SW, Eagle TM, Maddox JF, Mackman N, et al. Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice. Hepatology. 2007; 46:1177–1186. [PubMed: 17654741]
19. Sullivan BP, Kassel KM, Jone A, Flick MJ, Luyendyk JP. Fibrin(ogen)-independent role of plasminogen activators in acetaminophen-induced liver injury. The American journal of pathology. 2012; 180:2321–2329. [PubMed: 22507835]
20. Willingham AK, Matschiner JT. The activation of factor X by hepatocyte plasma membranes. Cellular and molecular biology. 1989; 35:421–429. [PubMed: 2611830]
21. Gustafson EK, Elgue G, Hughes RD, Mitry RR, Sanchez J, Haglund U, et al. The instant blood-mediated inflammatory reaction characterized in hepatocyte transplantation. Transplantation. 2011; 91:632–638. [PubMed: 21289595]
22. Stephenne X, Vosters O, Najimi M, Beuneu C, Dung KN, Wijns W, et al. Tissue factor-dependent procoagulant activity of isolated human hepatocytes: relevance to liver cell transplantation. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2007; 13:599–606.
23. Sullivan BP, Kopec AK, Joshi N, Cline H, Brown JA, Bishop SC, et al. Hepatocyte tissue factor activates the coagulation cascade in mice. Blood. 2013; 121:1868–1874. [PubMed: 23305736]
24. Michelotti GA, Xie G, Swiderska M, Choi SS, Karaca G, Kruger L, et al. Smoothened is a master regulator of adult liver repair. The Journal of clinical investigation. 2013; 123:2380–2394. [PubMed: 23563311]
25. Rao LV, Kothari H, Pendurthi UR. Tissue factor: mechanisms of decryption. Frontiers in bioscience. 2012; 4:1513–1527.

26. Hoffman M, Colina CM, McDonald AG, Arepally GM, Pedersen L, Monroe DM. Tissue factor around dermal vessels has bound factor VII in the absence of injury. Journal of thrombosis and haemostasis : JTH. 2007; 5:1403–1408. [PubMed: 17425666]
27. Boyer JL. Bile formation and secretion. Comprehensive Physiology. 2013; 3:1035–1078. [PubMed: 23897680]
28. Kassel KM, Owens AP 3rd, Rockwell CE, Sullivan BP, Wang R, Tawfik O, et al. Proteaseactivated receptor 1 and hematopoietic cell tissue factor are required for hepatic steatosis in mice fed a Western diet. The American journal of pathology. 2011; 179:2278–2289. [PubMed: 21907177]
29. Sullivan BP, Weinreb PH, Violette SM, Luyendyk JP. The coagulation system contributes to alphaVbeta6 integrin expression and liver fibrosis induced by cholestasis. The American journal of pathology. 2010; 177:2837–2849. [PubMed: 21037076]
30. Stephenne X, Nicastro E, Eeckhoudt S, Hermans C, Nyabi O, Lombard C, et al. Bivalirudin in combination with heparin to control mesenchymal cell procoagulant activity. PloS one. 2012; 7:e42819. [PubMed: 22900053]
31. Luyendyk JP, Flanagan KC, Williams CD, Jaeschke H, Slusser JG, Mackman N, et al. Tissue factor contributes to neutrophil CD11b expression in alpha-naphthylisothiocyanate-treated mice. Toxicology and applied pharmacology. 2011; 250:256–262. [PubMed: 21070799]
32. Kato J, Okamoto T, Motoyama H, Uchiyama R, Kirchhofer D, Van Rooijen N, et al. Interferon-gamma-mediated tissue factor expression contributes to T-cell-mediated hepatitis through induction of hypercoagulation in mice. Hepatology. 2013; 57:362–372. [PubMed: 22936459]
33. Hammad MA, Abdel-Bakky MS, Walker LA, Ashfaq MK. Tissue factor antisense deoxyoligonucleotide prevents monocrotaline/LPS hepatotoxicity in mice. Journal of applied toxicology : JAT. 2013; 33:774–783. [PubMed: 22407844]
34. Luyendyk JP, Sullivan BP, Guo GL, Wang R. Tissue factor-deficiency and protease activated receptor-1-deficiency reduce inflammation elicited by diet-induced steatohepatitis in mice. The American journal of pathology. 2010; 176:177–186. [PubMed: 20008134]

Table 1

Role of TF in models of liver injury and disease.

Reference	Authors	Liver injury/disease model	TF deficiency model	Outcome of TF deficiency
[18]	Ganey *et al.* 2007	Acetaminophen hepatotoxicity	Low TF mice	↓Coagulation ↓Liver injury
[23]	Sullivan *et al.* 2013	Acetaminophen hepatotoxicity	$TF^{flox/flox}$/ Alb-Cre mice	↓Coagulation
[15]	Luyendyk *et al.* 2009	α-naphthylisothiocyanate hepatotoxicity	Low TF mice	↓Coagulation ↓Liver injury
[31]	Luyendyk *et al.* 2011	α-naphthylisothiocyanate hepatotoxicity	$TF^{flox/flox}$/ LysM-Cre mice	↓Neutrophil activation
[29]	Sullivan *et al.* 2010	α-naphthylisothiocyanate-induced liver fibrosis	TF+/− mice	↓Coagulation ↓Liver fibrosis
[32]	Kato *et al.* 2013	Concanavalin A hepatotoxicity	Neutralizing antibody (1H1)	↓Coagulation ↓Liver apoptosis/injury
[33]	Hammad *et al.* 2013	Monocrotaline/LPS hepatotoxicity	TF ASO	↓Coagulation ↓Liver injury
[34]	Luyendyk *et al.* 2010	Steatohepatitis (MCD diet)	Low TF mice	↓Coagulation ↓Liver injury ↓Liver inflammation
[28]	Kassel *et al.* 2011	Steatosis (Western diet)	Hematopoietic cell TF deficiency	↓Coagulation ↓Steatosis/triglycerides ↓Liver inflammation