Von Willebrand factor deficiency reduces liver fibrosis in mice

Nikita Joshi[1,3], **Anna K Kopec**[2,3], **Jessica L Ray**[2], **Holly Cline-Fedewa**[2], **Dafna J Groeneveld**[4], **Ton Lisman**[4], and **James P Luyendyk**[1,2,3]

[1]Department of Pharmacology & Toxicology, Michigan State University, East Lansing, Michigan
[2]Department of Pathobiology & Diagnostic Investigation, Michigan State University, East Lansing, Michigan [3]Institute for Integrative Toxicology, Michigan State University, East Lansing, Michigan
[4]Surgical Research Laboratory, Department of Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Abstract

Liver diseases are associated with complex changes in the hemostatic system and elevated levels of the platelet-adhesive protein Von Willebrand factor (VWF) are reported in patients with acute and chronic liver damage. Although elevated levels of VWF are associated with fibrosis in the general population, the role of VWF in acute and chronic liver injury has not been examined in depth in experimental settings. We tested the hypothesis that VWF deficiency inhibits experimental liver injury and fibrosis. Wild-type (WT) and VWF-deficient mice were challenged with carbon tetrachloride (CCl_4) and the impact of VWF deficiency on acute liver injury and chronic liver fibrosis was determined. VWF deficiency did not significantly affect acute CCl_4-induced hepatocellular necrosis in mice. Chronic CCl_4 challenge, twice weekly for 6 weeks, significantly increased hepatic stellate cell activation and collagen deposition in livers of WT mice. Interestingly, hepatic induction of several profibrogenic and stellate cell activation genes was attenuated in VWF-deficient mice. Moreover, birefringent sirius red staining (indicating type I and III collagens) and type I collagen immunofluorescence indicated a reduction in hepatic collagen deposition in CCl_4-exposed VWF-deficient mice compared to CCl_4-exposed WT mice. The results indicate that VWF deficiency attenuates chronic CCl_4-induced liver fibrosis without affecting acute hepatocellular necrosis. The results are the first to demonstrate that VWF deficiency reduces the progression of liver fibrosis, suggesting a mechanistic role of elevated plasma VWF levels in cirrhosis.

Keywords

Liver cirrhosis; thrombosis; hemostatic disorders

---

[1]To whom correspondence should be addressed: James P. Luyendyk, Department of Pathobiology and Diagnostic Investigation, Michigan State University, 1129 Farm Lane, 253 Food Safety and Toxicology Building, East Lansing, MI 48824, Phone: (517) 884-2057, Fax: (517) 884-2012; luyendyk@cvm.msu.edu.

Introduction

Complex changes in the hemostatic system are evident in patients with acute and chronic liver injury (Lisman *et al.*, 2010; Tripodi and Mannucci, 2011). Chronic liver disease can result in replacement of functional liver mass with extracellular matrix, primarily collagens. The outcome of this process is cirrhosis, which can predispose patients to thrombosis, and may also contribute to progression of liver disease itself (Jairath and Burroughs, 2013). Among the hemostatic molecules disturbed in both acute and chronic liver disease is Von Willebrand factor (VWF), a glycoprotein that functions to bridge platelets with an exposed collagen surface. Several studies report that plasma levels of VWF (Reuken *et al.*, 2015) are increased in patients with acute liver failure, as well as cirrhosis, with high levels predictive of poor outcome in this patient population (La Mura *et al.*, 2011; Ferlitsch *et al.*, 2012; Hugenholtz *et al.*, 2013; Maieron *et al.*, 2014; Kalambokis *et al.*, 2016). Suggesting a functional connection, VWF levels are associated with the severity of liver fibrosis in the general population (Plompen *et al.*, 2015). Thus, there is strong evidence in patients linking elevated VWF levels with both acute liver injury and with consequences of chronic liver disease such as cirrhosis.

Despite the association between VWF levels and cirrhosis in patients, the precise nature of this association is not understood. Increased plasma VWF levels in cirrhosis are most likely ascribed to endothelial cell activation, induction of hepatic expression (Ferro *et al.*, 1996; Hollestelle *et al.*, 2004), or perhaps reduced liver-mediated clearance (Lisman *et al.*, 2006). That is, elevated plasma VWF levels may be a biomarker of elevated hepatic expression, driven by liver disease itself. However, strong evidence indicates that components of the hemostatic system actually drive the activation of hepatic stellate cells in experimental liver fibrosis (Anstee *et al.*, 2011; Tripodi *et al.*, 2011). Indeed, several studies illustrate the potential for coagulation proteases, both by activation of intracellular signaling and formation of intrahepatic microthrombi, to directly exacerbate liver fibrosis (Anstee *et al.*, 2008; Rullier *et al.*, 2008; Sullivan *et al.*, 2010). Importantly, the association of VWF with cirrhosis, while inferred by clinical association studies, has not been examined in experimental studies to determine whether VWF plays a direct mechanistic role in liver fibrosis.

To address this critical gap in the literature, we tested the hypothesis that VWF directly contributes to experimental liver fibrosis by employing an established mouse model of genetic VWF deficiency. Using this tool, we determined the effect of VWF deficiency on acute carbon tetrachloride (CCl_4)-induced liver injury and on liver fibrosis driven by chronic CCl_4 challenge. Collectively, these studies represent the first published report describing the impact of VWF deficiency in experimental settings of acute and chronic liver injury.

Materials and Methods

Mice

Congenic C57Bl/6J VWF$^{-/-}$ mice were obtained from Jackson Laboratory (Bar Harbor, ME), were maintained by homozygous breeding, and have been described previously (Denis *et al.*, 1998). Age- and sex-matched wild-type C57Bl/6J mice were purchased from Jackson

Laboratory and allowed to acclimate in the same facility for at least 3 weeks prior to experimental challenge. Male and female mice used for these studies were between the ages of 8–14 weeks. Mice were housed in an AAALAC-accredited facility and allowed *ad libitum* access to purified drinking water and rodent chow. All animal procedures were approved by the Michigan State University IACUC.

Acute and chronic CCl$_4$ challenge

10% CCl$_4$ in corn oil vehicle was administered to mice by intraperitoneal injection (10 ml/kg). For acute liver injury studies, samples were collected 48 or 72 hours after CCl$_4$ challenge. Chronic CCl$_4$-induced liver fibrosis was induced by administration of CCl$_4$ every 3–4 days (i.e., every Tuesday and Friday) for 6 weeks. Blood and liver samples were collected three days after the last injection of CCl$_4$.

Histopathology, immunohistochemistry and immunofluorescence

Hematoxylin and eosin (H&E), sirius red, alpha-smooth muscle actin (α-SMA), proliferating cell nuclear antigen (PCNA) and type I collagen stains were performed and analyzed as described previously (Sullivan *et al.*, 2010; Joshi *et al.*, 2016; Kopec *et al.*, 2016). Necrosis area was calculated as described previously (Joshi *et al.*, 2016).

Determination of VWF, lipid peroxidation, and serum ALT

Plasma VWF antigen levels were determined by ELISA as described previously (Groeneveld *et al.*, 2016) and pooled normal human plasma was utilized to develop a standard curve. Thiobarbituric acid reactive substances (TBARS) were determined using a commercially available kit (Cayman Chemical, Ann Arbor, MI). Serum alanine aminotransferase (ALT) activity was determined using commercial reagents (Thermo Fisher, Waltham, MA).

RNA isolation, cDNA synthesis, and quantitative real-time PCR (qPCR)

Detection and quantification of select mRNAs by SYBR Green qPCR was performed using primers described previously (Joshi *et al.*, 2016).

Statistical analyses

Comparison of two groups was performed using Student's t-test. Comparison of three or more groups was performed using one- or two-way analysis of variance (ANOVA), as appropriate, and Student-Newman-Keul's *post hoc* test. The criterion for statistical significance was P<0.05.

Results

Effect of VWF deficiency on acute CCl$_4$-induced liver injury

Plasma VWF levels were significantly increased in CCl$_4$-exposed wild-type mice at 48 and 72 hours (Fig. 1A). Complete VWF deficiency did not affect lipid peroxidation at 48 hours, a surrogate marker of CCl$_4$ metabolism (Fig. 1B). Both male and female mice were challenged with CCl$_4$ and there was no apparent sex-dependent difference in liver injury, in agreement with prior studies (Bhathal *et al.*, 1983). Serum ALT activity, an indicator of

hepatocellular injury, was significantly increased in CCl_4-exposed male and female $VWF^{-/-}$ mice compared to wild-type mice at 48 hours (Fig. 1C). However, this did not correspond to a significant increase in centrilobular liver necrosis in CCl_4-exposed $VWF^{-/-}$ mice (Fig. 1D–E). Serum ALT activity and hepatocellular necrosis were reduced at 72 hours, regardless of genotype (Fig. 1C–E). Moreover, the number of PCNA-positive hepatocytes at both 48 and 72 hours was not significantly affected by VWF deficiency (Fig. 1F–G).

VWF deficiency reduces α-smooth muscle actin and profibrogenic gene induction in liver after chronic CCl_4 challenge

Plasma VWF levels were significantly increased in wild-type mice after chronic (6 weeks) CCl_4 challenge (Fig. 2A). Serum ALT activity was increased modestly in wild-type mice challenged with chronic CCl_4 and this was unaffected by VWF deficiency (Fig. 2B). As anticipated, chronic CCl_4 challenge in wild-type mice was associated with increased hepatic stellate cell transition to myofibroblasts, marked by significant increases in hepatic *Acta2* and corresponding α-SMA protein expression (Fig. 2C–E). Interestingly, induction of *Acta2* and α-SMA protein was significantly reduced in $VWF^{-/-}$ mice (Fig. 2C–E). In agreement with these measures suggesting reduced stellate cell activation, induction of additional profibrogenic genes, *Tgfb1*, *Tgfb2* and *Col1a1*, was also significantly reduced in CCl_4-exposed $VWF^{-/-}$ mice compared to CCl_4-exposed wild-type mice (Fig. 2F–H).

VWF deficiency reduces liver fibrosis after chronic CCl_4 challenge

Hepatic stellate cells are well-recognized as the primary cellular source of extracellular matrix (e.g., collagens) in liver fibrosis; thus, we utilized multiple approaches to examine the effect of VWF deficiency on liver fibrosis. First, we quantified hepatic collagen deposition by examining sirius red-stained sections under polarized light, an approach that isolates the birefringent property of type I and III collagen (Junqueira *et al.*, 1978; Street *et al.*, 2014). Collagen deposition significantly increased in CCl_4-challenged wild-type mice, but this increase was significantly reduced in CCl_4-challenged $VWF^{-/-}$ mice (Fig. 3A–B). To validate the reduction in collagen deposition in $VWF^{-/-}$ mice, we evaluated type I collagen deposits by a direct immunolabeling approach. By this method, type I collagen deposits significantly increased in livers of wild-type CCl_4-challenged mice, and this was significantly attenuated in CCl_4-challenged $VWF^{-/-}$ mice (Fig. 3C–D). Altogether, the results indicate that VWF deficiency significantly reduces collagen deposition in a well-validated experimental setting of liver fibrosis.

Discussion

Increased plasma VWF levels are closely associated with poor outcome in patients with cirrhosis, and VWF levels are associated with fibrosis in the general population (La Mura *et al.*, 2011; Ferlitsch *et al.*, 2012; Maieron *et al.*, 2014; Plompen *et al.*, 2015; Kalambokis *et al.*, 2016). Building on this clinical association, we provide the first experimental evidence that VWF directly contributes to liver fibrosis. Similar to patients with liver disease, we observed that both acute and chronic experimental liver injury elevated plasma VWF levels. Whereas VWF deficiency had minimal effect on acute CCl_4 hepatotoxicity, activation of hepatic stellate cells, suggested by α-SMA expression, and hepatic collagen deposition

triggered by chronic CCl_4 challenge were reduced in VWF-deficient mice. These studies demonstrate a causal link between VWF and fibrosis, an important observation building on the clinical association between VWF and cirrhosis in patients. This suggests that in addition to serving as a biomarker of cirrhosis severity, elevated plasma VWF levels offer insight into how components of the hemostatic system contribute to fibrosis.

The mechanism whereby VWF contributes to experimental liver fibrosis is not completely understood. The hemostatic system is altered in patients with cirrhosis, predisposing patients to thrombosis (Tripodi, 2015). Experimental studies also indicate that components of the hemostatic system drive liver fibrosis (Anstee *et al.*, 2011). Published clinical studies support this hypothesis (Villa *et al.*, 2012; Poujol-Robert *et al.*, 2014) and additional clinical trials are underway to examine the effect of anticoagulants in cirrhosis (e.g., NCT02271295, NCT02643212). Elevated VWF levels are one of the reported hemostatic alterations in patients with cirrhosis. VWF binding to the GPIb/IX/V complex, and to collagen, bridges platelets to exposed collagen surfaces (Ruggeri, 2003). VWF deficiency predisposes to bleeding in humans and also reduces thrombus formation in experimental models (Denis *et al.*, 1998; Leebeek and Eikenboom, 2016). Thus, one mechanism whereby VWF deficiency could reduce fibrosis in experimental systems is by reducing platelet accumulation/function. Limitation in next level mechanistic studies examining this possibility is that interaction between VWF and platelets is rapid and may be missed without extensive time course investigation. It is also possible that VWF-platelet interaction drives liver fibrosis through formation of thrombi in extrahepatic vessels.

Anticoagulants have emerged as a candidate therapeutic class to reduce cirrhosis and have been shown to reduce disease progression in both experimental animal models as well as in humans (Ganey *et al.*, 2007; Fujita *et al.*, 2008; Anstee *et al.*, 2011; Villa *et al.*, 2012). Although currently available anticoagulants show promising results, the risk of bleeding is still a major drawback. Especially in patients with impaired liver function, which is associated with a complex coagulopathy, bleeding risk dramatically limits therapeutic potential of existing drugs (Lisman *et al.*, 2013). Agents inhibiting VWF function by blocking the VWF-GPIbα or VWF-collagen interaction have been developed in the past decade (De Meyer *et al.*, 2012). These inhibitors include monoclonal antibodies against VWF and DNA aptamers that act like chemical antibodies by binding to VWF with high affinity. Preclinical and clinical studies have already demonstrated the antithrombotic potential of such inhibitors (reviewed in (De Meyer *et al.*, 2012). Moreover, clinical testing in animal models and healthy individuals points towards a profoundly lower bleeding risk with equal or even higher efficacy as compared to established anti-platelet drugs (Kageyama *et al.*, 1997; Kageyama *et al.*, 2002; Ulrichts *et al.*, 2011). Our results indicate that VWF directly contributes to liver fibrosis. Thus, targeting VWF functionality with these monoclonal antibodies or DNA aptamers could therefore be a novel therapeutic approach in the treatment of chronic liver disease.

In summary, we report that VWF deficiency significantly attenuates the cellular profibrogenic response and fibrosis in experimental chronic liver injury. The results solidify the role of VWF as a causal factor in liver fibrosis, building on strong clinical evidence of an association between elevated VWF levels with cirrhosis. Additional mechanistic studies

defining the mechanism whereby VWF exacerbates experimental fibrosis are likely to reveal novel links between the hemostatic system and cirrhosis. Such studies have the potential to identify specific VWF functions as putative therapeutic targets for liver cirrhosis.

Acknowledgments

References

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]

Anstee QM, Goldin RD, Wright M, Martinelli A, Cox R, Thursz MR. Coagulation status modulates murine hepatic fibrogenesis: implications for the development of novel therapies. Journal of thrombosis and haemostasis: JTH. 2008; 6:1336–1343. [PubMed: 18485088]

Bhathal PS, Rose NR, Mackay IR, Whittingham S. Strain differences in mice in carbon tetrachloride-induced liver injury. British journal of experimental pathology. 1983; 64:524–533. [PubMed: 6639871]

De Meyer SF, Stoll G, Wagner DD, Kleinschnitz C. von Willebrand factor: an emerging target in stroke therapy. Stroke. 2012; 43:599–606. [PubMed: 22180250]

Denis C, Methia N, Frenette PS, Rayburn H, Ullman-Cullere M, Hynes RO, Wagner DD. A mouse model of severe von Willebrand disease: defects in hemostasis and thrombosis. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95:9524–9529. [PubMed: 9689113]

Ferlitsch M, Reiberger T, Hoke M, Salzl P, Schwengerer B, Ulbrich G, Payer BA, Trauner M, Peck-Radosavljevic M, Ferlitsch A. von Willebrand factor as new noninvasive predictor of portal hypertension, decompensation and mortality in patients with liver cirrhosis. Hepatology. 2012; 56:1439–1447. [PubMed: 22532296]

Ferro D, Quintarelli C, Lattuada A, Leo R, Alessandroni M, Mannucci PM, Violi F. High plasma levels of von Willebrand factor as a marker of endothelial perturbation in cirrhosis: relationship to endotoxemia. Hepatology. 1996; 23:1377–1383. [PubMed: 8675154]

Fujita K, Nozaki Y, Wada K, Yoneda M, Endo H, Takahashi H, Iwasaki T, Inamori M, Abe Y, Kobayashi N, Kirikoshi H, Kubota K, Saito S, Nagashima Y, Nakajima A. Effectiveness of antiplatelet drugs against experimental non-alcoholic fatty liver disease. Gut. 2008; 57:1583–1591. [PubMed: 18596193]

Ganey PE, Luyendyk JP, Newport SW, Eagle TM, Maddox JF, Mackman N, Roth RA. Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice. Hepatology. 2007; 46:1177–1186. [PubMed: 17654741]

Groeneveld DJ, Alkozai EM, Adelmeijer J, Porte RJ, Lisman T. Balance between von Willebrand factor and ADAMTS13 following major partial hepatectomy. The British journal of surgery. 2016

Hollestelle MJ, Geertzen HG, Straatsburg IH, van Gulik TM, van Mourik JA. Factor VIII expression in liver disease. Thrombosis and haemostasis. 2004; 91:267–275. [PubMed: 14961153]

Hugenholtz GC, Adelmeijer J, Meijers JC, Porte RJ, Stravitz RT, Lisman T. An unbalance between von Willebrand factor and ADAMTS13 in acute liver failure: implications for hemostasis and clinical outcome. Hepatology. 2013; 58:752–761. [PubMed: 23468040]

Jairath V, Burroughs AK. Anticoagulation in patients with liver cirrhosis: complication or therapeutic opportunity? Gut. 2013; 62:479–482. [PubMed: 23300140]

Joshi N, Kopec AK, Ray JL, Cline-Fedewa H, Nawabi A, Schmitt T, Nault R, Zacharewski TR, Rockwell CE, Flick MJ, Luyendyk JP. Fibrin deposition following bile duct injury limits fibrosis through an alphaMbeta2-dependent mechanism. Blood. 2016; 127:2751–2762. [PubMed: 26921287]

Junqueira LC, Cossermelli W, Brentani R. Differential staining of collagens type I, II and III by Sirius Red and polarization microscopy. Archivum histologicum Japonicum = Nihon soshikigaku kiroku. 1978; 41:267–274. [PubMed: 82432]

Kageyama S, Yamamoto H, Nagano M, Arisaka H, Kayahara T, Yoshimoto R. Anti-thrombotic effects and bleeding risk of AJvW-2, a monoclonal antibody against human von Willebrand factor. British journal of pharmacology. 1997; 122:165–171. [PubMed: 9298543]

Kageyama S, Yamamoto H, Nakazawa H, Matsushita J, Kouyama T, Gonsho A, Ikeda Y, Yoshimoto R. Pharmacokinetics and pharmacodynamics of AJW200, a humanized monoclonal antibody to von Willebrand factor, in monkeys. Arteriosclerosis, thrombosis, and vascular biology. 2002; 22:187–192.

Kalambokis GN, Oikonomou A, Christou L, Kolaitis NI, Tsianos EV, Christodoulou D, Baltayiannis G. von Willebrand factor and procoagulant imbalance predict outcome in patients with cirrhosis and thrombocytopenia. Journal of hepatology. 2016; 65:921–928. [PubMed: 27297911]

Kopec AK, Joshi N, Cline-Fedewa H, Wojcicki AV, Ray JL, Sullivan BP, Froehlich JE, Johnson BF, Flick MJ, Luyendyk JP. Fibri(nogen) drives repair after acetaminophen-induced liver injury via leukocyte alphaMbeta2 integrin-dependent upregulation of MMP12. Journal of hepatology. 2016

La Mura V, Reverter JC, Flores-Arroyo A, Raffa S, Reverter E, Seijo S, Abraldes JG, Bosch J, Garcia-Pagan JC. Von Willebrand factor levels predict clinical outcome in patients with cirrhosis and portal hypertension. Gut. 2011; 60:1133–1138. [PubMed: 21427197]

Leebeek FW, Eikenboom JC. Von Willebrand's Disease. The New England journal of medicine. 2016; 375:2067–2080. [PubMed: 27959741]

Lisman T, Bongers TN, Adelmeijer J, Janssen HL, de Maat MP, de Groot PG, Leebeek FW. Elevated levels of von Willebrand Factor in cirrhosis support platelet adhesion despite reduced functional capacity. Hepatology. 2006; 44:53–61. [PubMed: 16799972]

Lisman T, Caldwell SH, Burroughs AK, Northup PG, Senzolo M, Stravitz RT, Tripodi A, Trotter JF, Valla DC, Porte RJ, Coagulation in Liver Disease Study, G. Hemostasis and thrombosis in patients with liver disease: the ups and downs. Journal of hepatology. 2010; 53:362–371. [PubMed: 20546962]

Lisman T, Kamphuisen PW, Northup PG, Porte RJ. Established and new-generation antithrombotic drugs in patients with cirrhosis - possibilities and caveats. Journal of hepatology. 2013; 59:358–366. [PubMed: 23548197]

Maieron A, Salzl P, Peck-Radosavljevic M, Trauner M, Hametner S, Schofl R, Ferenci P, Ferlitsch M. Von Willebrand Factor as a new marker for non-invasive assessment of liver fibrosis and cirrhosis in patients with chronic hepatitis C. Alimentary pharmacology & therapeutics. 2014; 39:331–338. [PubMed: 24308724]

Plompen EP, Darwish Murad S, Hansen BE, Loth DW, Schouten JN, Taimr P, Hofman A, Uiterlinden AG, Stricker BH, Janssen HL, Leebeek FW. Prothrombotic genetic risk factors are associated with an increased risk of liver fibrosis in the general population: The Rotterdam Study. Journal of hepatology. 2015; 63:1459–1465. [PubMed: 26226452]

Poujol-Robert A, Boelle PY, Conti F, Durand F, Duvoux C, Wendum D, Paradis V, Mackiewicz V, Chazouilleres O, Corpechot C, Poupon R. Aspirin may reduce liver fibrosis progression: Evidence from a multicenter retrospective study of recurrent hepatitis C after liver transplantation. Clinics and research in hepatology and gastroenterology. 2014; 38:570–576. [PubMed: 25130796]

Reuken PA, Kussmann A, Kiehntopf M, Budde U, Stallmach A, Claus RA, Bruns T. Imbalance of von Willebrand factor and its cleaving protease ADAMTS13 during systemic inflammation superimposed on advanced cirrhosis. Liver international: official journal of the International Association for the Study of the Liver. 2015; 35:37–45. [PubMed: 25113276]

Ruggeri ZM. Von Willebrand factor, platelets and endothelial cell interactions. Journal of thrombosis and haemostasis: JTH. 2003; 1:1335–1342. [PubMed: 12871266]

Rullier A, Gillibert-Duplantier J, Costet P, Cubel G, Haurie V, Petibois C, Taras D, Dugot-Senant N, Deleris G, Bioulac-Sage P, Rosenbaum J. Protease-activated receptor 1 knockout reduces experimentally induced liver fibrosis. American journal of physiology Gastrointestinal and liver physiology. 2008; 294:G226–235. [PubMed: 17962354]

Street JM, Souza AC, Alvarez-Prats A, Horino T, Hu X, Yuen PS, Star RA. Automated quantification of renal fibrosis with Sirius Red and polarization contrast microscopy. Physiological reports. 2014; 2

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]

Tripodi A. Hemostasis abnormalities in cirrhosis. Current opinion in hematology. 2015; 22:406–412. [PubMed: 26203733]

Tripodi A, Anstee QM, Sogaard KK, Primignani M, Valla DC. Hypercoagulability in cirrhosis: causes and consequences. Journal of thrombosis and haemostasis: JTH. 2011; 9:1713–1723. [PubMed: 21729237]

Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. The New England journal of medicine. 2011; 365:147–156. [PubMed: 21751907]

Ulrichts H, Silence K, Schoolmeester A, de Jaegere P, Rossenu S, Roodt J, Priem S, Lauwereys M, Casteels P, Van Bockstaele F, Verschueren K, Stanssens P, Baumeister J, Holz JB. Antithrombotic drug candidate ALX-0081 shows superior preclinical efficacy and safety compared with currently marketed antiplatelet drugs. Blood. 2011; 118:757–765. [PubMed: 21576702]

Villa E, Camma C, Marietta M, Luongo M, Critelli R, Colopi S, Tata C, Zecchini R, Gitto S, Petta S, Lei B, Bernabucci V, Vukotic R, De Maria N, Schepis F, Karampatou A, Caporali C, Simoni L, Del Buono M, Zambotto B, Turola E, Fornaciari G, Schianchi S, Ferrari A, Valla D. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. Gastroenterology. 2012; 143:1253–1260 e1251. 1254. [PubMed: 22819864]

Highlights

- Plasma von Willebrand Factor (VWF) levels increase in experimental liver damage

- VWF contributes to liver myofibroblast activation in experimental fibrosis

- VWF deficiency reduces experimental fibrosis without affecting acute liver injury
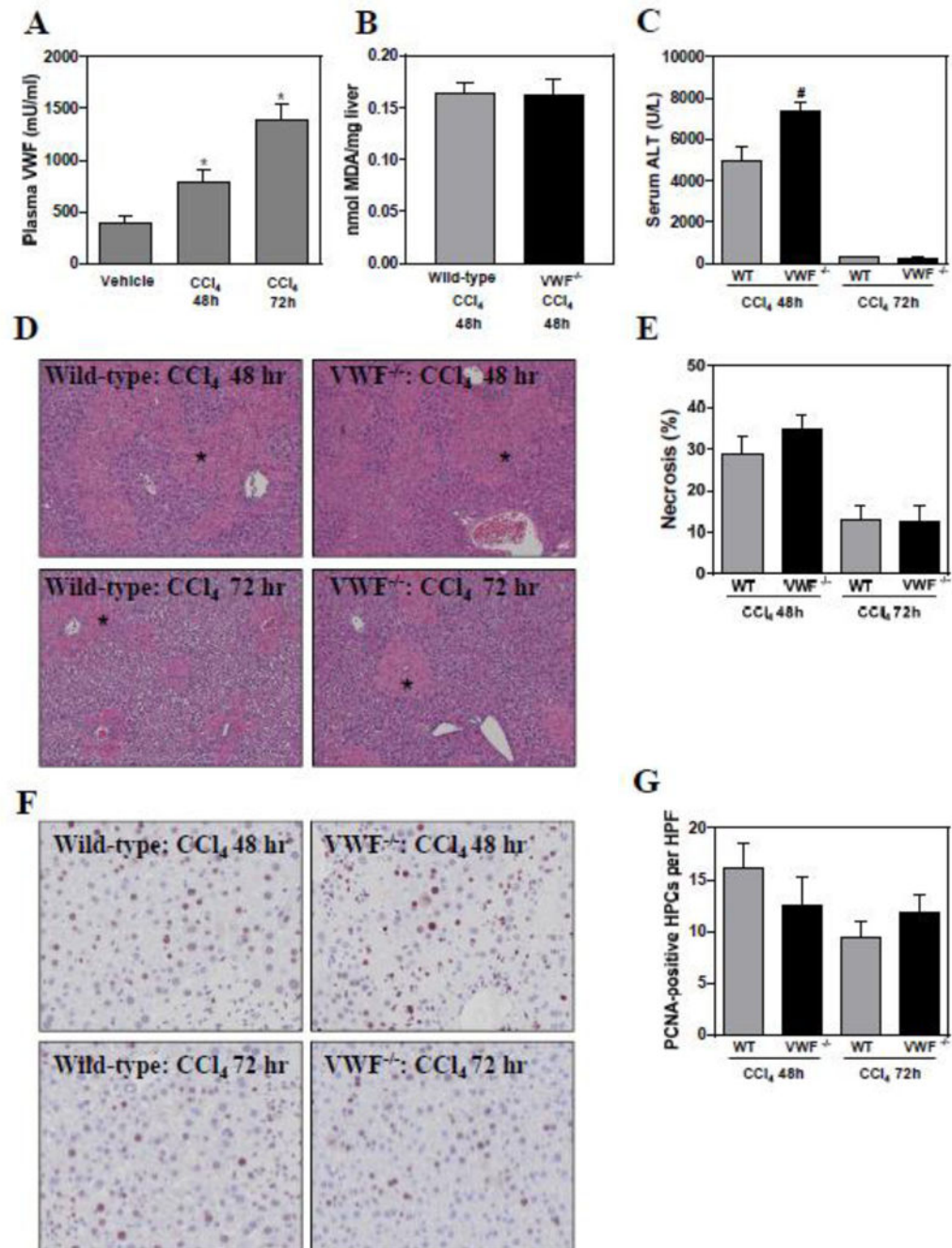
Figure 1.
Effect of VWF deficiency on acute CCl_4-induced liver injury. Male and female wild-type (WT) and VWF$^{-/-}$ mice were given carbon tetrachloride (CCl_4) dissolved to 10% in corn oil (10 ml/kg, ip) or vehicle (corn oil) and (A) plasma VWF concentration, (B) hepatic malondialdehyde (MDA) levels, and (C) serum alanine aminotransferase (ALT) activity were assessed 48 and 72 hours after CCl_4 challenge. (D) Representative photomicrographs (original magnification × 100) show areas of hepatocellular necrosis (stars) in H&E-stained liver sections. (E) Quantification of hepatocellular necrosis area as described in materials

and methods. (F) Representative photomicrographs depict immunohistochemical labeling of proliferating cell nuclear antigen (PCNA)-positive hepatocytes (brown), which were (G) quantified as described in materials and methods. Data are expressed as mean + SEM. N=6–11 mice per group. *Significantly different from vehicle-treated mice. #Significantly different from CCl_4-challenged WT mice at that time. $P < 0.05$.
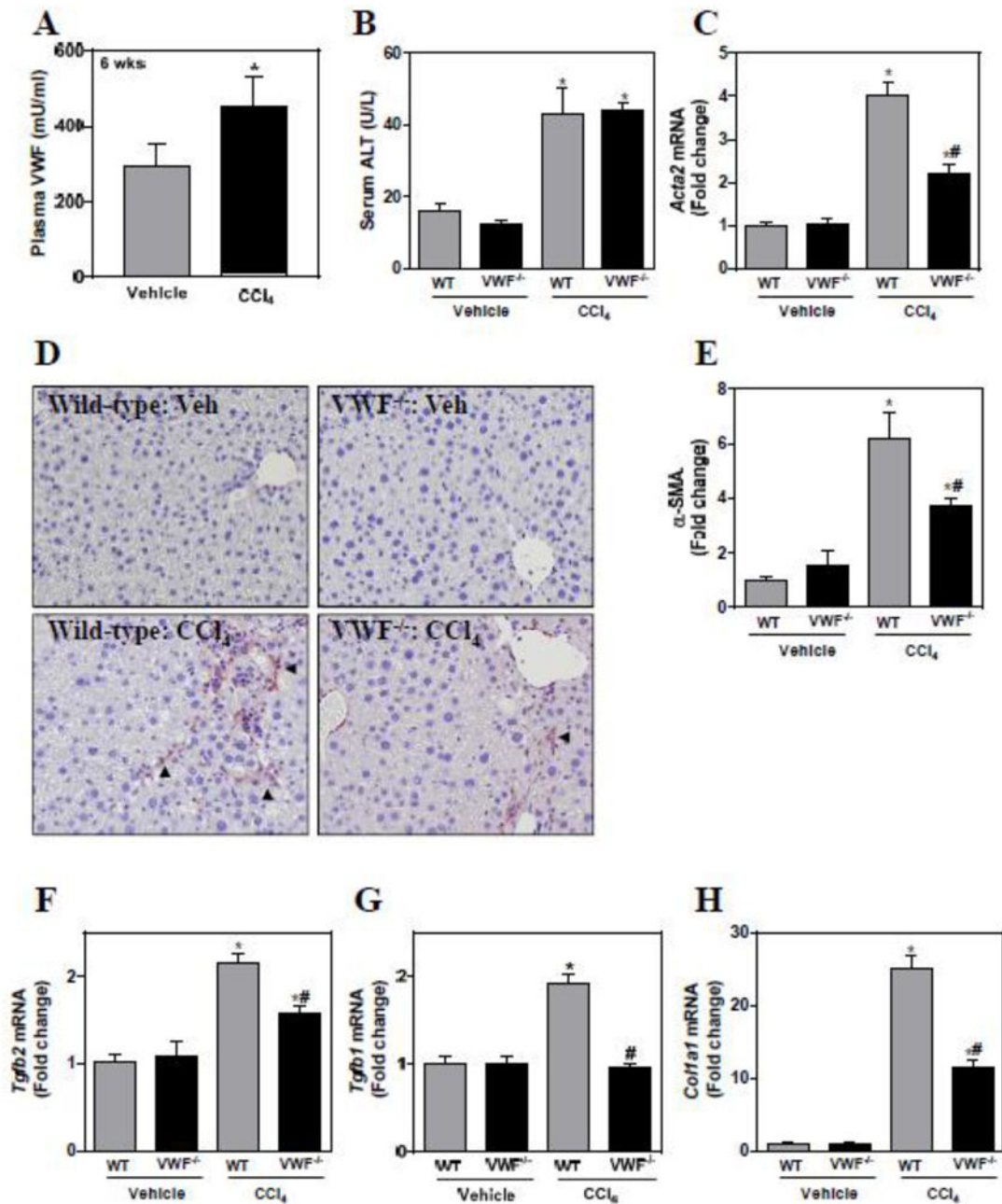
Figure 2.
Effect of VWF deficiency on profibrogenic changes in liver after chronic CCl$_4$ challenge. Female wild-type (WT) and VWF$^{-/-}$ mice were given carbon tetrachloride (CCl$_4$) dissolved to 10% in corn oil (10 ml/kg, ip) twice weekly for 6 weeks. (A) Plasma VWF concentration. (B) Serum alanine aminotransferase (ALT) activity. Hepatic levels of profibrogenic genes (C) *Acta2*, (F) *Tgfb2*, (G) *Tgfb1*, and (H) *Col1a1* were determined as described in materials and methods and expressed as fold-change vs. vehicle-treated mice of the same genotype. (D) Representative photomicrographs depict immunohistochemical labeling of alpha-smooth muscle actin (α-SMA) (arrowheads, brown) in liver sections. (E) The area of α-SMA staining was quantified as described in materials and methods. Data are expressed as mean +

SEM. N=5–8 mice per group for vehicle-treated mice and 16–20 for CCl$_4$-challenged mice. *Significantly different from vehicle-treated mice of the same genotype. #Significantly different from CCl$_4$-challenged WT mice. P<0.05.
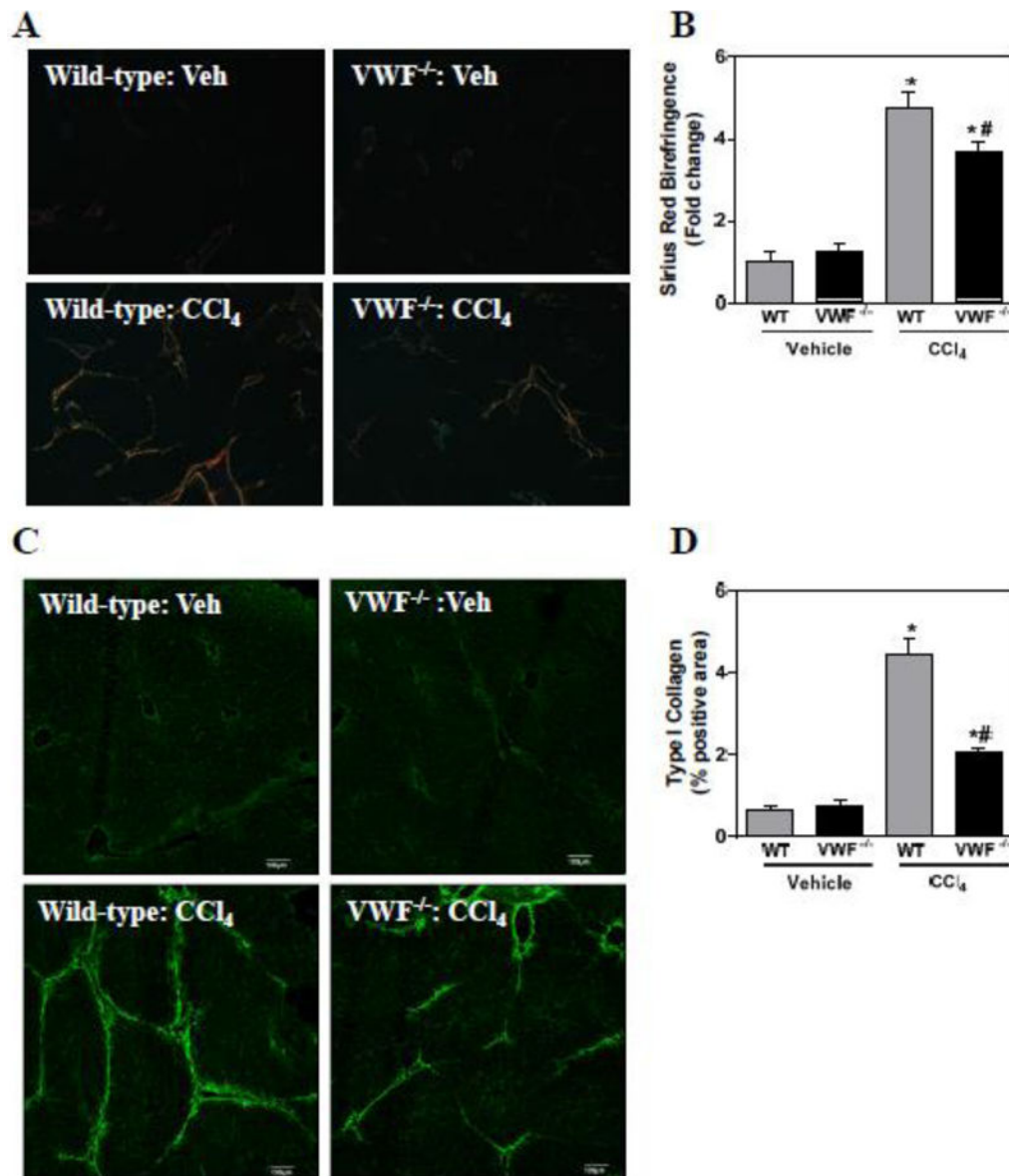
Figure 3.
Effect of VWF deficiency on liver fibrosis after chronic CCl$_4$ challenge. Female wild-type (WT) and VWF$^{-/-}$ mice were given carbon tetrachloride (CCl$_4$) dissolved to 10% in corn oil (10 ml/kg, ip) twice weekly for 6 weeks. (A–B) Representative photomicrographs and quantification of sirius red birefringence in liver sections visualized under polarized light. (C–D) Representative photomicrographs and quantification of type I collagen immunofluorescent staining of frozen liver sections. Data are expressed as mean + SEM. For panel B, N=5–8 mice per group for vehicle-treated mice and 16–20 for CCl$_4$-challenged mice. N=3 mice per group for panel D. *Significantly different from vehicle-treated mice of the same genotype. #Significantly different from CCl$_4$-challenged WT mice. P<0.05.