Minireview

Alcoholic liver disease and the potential role of plasminogen activator inhibitor-1 and fibrin metabolism

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Abstract

Plasminogen activator inhibitor-1 (PAI-1) is a major player in fibrinolysis due to its classical role of inhibiting plasminogen activators. Although increased fibrinolysis is common in alcoholic cirrhosis, decreased fibrinolysis (driven mostly by elevated levels of PAI-1) is common during the development of alcoholic liver disease (ALD). However, whether or not PAI-1 plays a causal role in the development of early ALD was unclear. Recent studies in experimental models have suggested that PAI-1 may contribute to the development of early (steatosis), intermediate (steatohepatitis) and late (fibrosis) stages of ALD. For example, fatty liver owing to both acute and chronic ethanol was blunted by the genetic inhibition of PAI-1. This effect of targeting PAI-1 appears to be mediated, at least in part, by an increase in very low-density lipoprotein (VLDL) synthesis in the genetic absence of this acute phase protein. Results from a two-hit model employing ethanol and lipopolysaccharide administration suggest that PAI-1 plays a critical role in hepatic inflammation, most likely due to its ability to cause fibrin accumulation, which subsequently sensitizes the liver to ensuing damaging insults. Lastly, the role of PAI-1 in hepatic fibrosis is less clear and appears that PAI-1 may serve a dual role in this pathological change, both protective (enhancing regeneration) and damaging (blocking matrix degradation). In summary, results from these studies suggest that PAI-1 may play multiple roles in the various stages of ALD, both protective and damaging. The latter effect is mediated by its influence on steatosis (i.e. decreasing VLDL synthesis), inflammation (i.e. impairing fibrinolysis) and fibrosis (i.e. blunting matrix degradation), whereas the former is mediated by maintaining hepatocyte division after an injury.

Keywords: coagulation cascade, ethanol, plasminogen system

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Introduction

Alcoholic liver disease: the need for new therapies

The risk of alcohol-induced liver disease (ALD) increases with the amount and length of consumption. ALD ranks among the major causes of morbidity and mortality in the world,[1] and affects millions of patients worldwide each year. From 1985 to 1992, the treatment of people afflicted with ALD cost over $148 billion in the USA.[2] Progression of the disease is well characterized and is actually a spectrum of liver diseases, which ranges initially from simple steatosis, to inflammation and necrosis (steatohepatitis), to fibrosis and cirrhosis.

A major focus in ALD therapy is to treat the decompensation associated with the disease. Indeed, the sequelae of a failing liver (e.g. ascites, portal hypertension and hepatorenal syndrome) are generally what causes death in end-stage liver disease.[3] Although the successful treatment of these secondary effects prolongs the life of ALD patients, this therapy is only palliative. Furthermore, since underlying cirrhosis greatly increases the risk of developing hepatocellular carcinoma (HCC),[4] success in maintaining 'stable cirrhotics' may translate into an increase in the incidence of HCC. Indeed, HCC incidence is increasing in the USA and in Europe.[5] Once a person develops HCC, the survival rate is almost nil.[6] The high mortality rate of cirrhosis/HCC further emphasizes the need for a therapy that can prevent the progression of ALD before cirrhosis develops.

Another effective approach to treat ALD is to encourage abstinence. Indeed, early stage alcohol-induced pathology (steatosis) is readily reversible with abstinence from alcohol.[7,8] Abstinence is also effective in the more severe stages of ALD, and increases survival even in decompensated cirrhotics.[3,9] However, the high rate of recidivism in alcoholics prevents abstinence from being generally effective for ALD. Liver transplantation is also an effective means to treat ALD; however, issues such as organ shortage and costs limit this therapy.[3]

Although the progression of alcohol-induced liver injury is well characterized, there is no universally accepted therapy available to halt or reverse this process in humans. Therefore, there is increasing focus on understanding the biochemical changes responsible for the development and progression of ALD. With better understanding of the mechanism(s) and risk factors that mediate the initiation and progression of this disease, rational targeted therapy can be developed to treat or prevent it in the clinics. The purpose of this review is to summarize the known mechanisms of damage in the major stages of the disease and to highlight recent work investigating the potential role of plasminogen activator inhibitor-1 (PAI-1) and fibrin metabolism in mediating this disease.

The natural history of ALD

Alcoholic steatosis (fatty liver)

The first and most common hepatic change caused by alcohol consumption is steatosis, or fatty liver. Fat accumulation can be both macrovesicular (having one large fat droplet per hepatocyte and lateral displacement of the nucleus) or microvesicular (many small fat droplets per hepatocyte).[10] As mentioned above, steatosis is rapidly and readily reversible upon cessation of alcohol consumption. Steatosis can also be clinically 'silent', and exist in the absence of increases in any other index of liver damage (e.g. plasma transaminases). For these reasons, steatosis was originally viewed as an inert pathology in ALD (and in other fatty liver diseases). However, more recent studies have suggested that blunting or preventing steatosis could help attenuate the progression of ALD,[11] which challenges the assumption that steatosis is an inert pathology. Hepatic fat accumulation can invoke metabolic changes that sensitize the liver to further injury (see below). Therefore, a full understanding of how alcohol induces steatosis could be key in preventing the further stages of ALD.

One mechanism by which alcohol exposure causes steatosis is directly via alcohol metabolism. Concentrations of alcohol can easily reach the millimolar range in the portal/hepatic circulation during alcohol consumption. In the process of metabolizing ethanol to acetate, two equivalents of reduced NADH are generated per equivalent of ethanol oxidized. This metabolism robustly increases the ratio of NADH:NAD^+ within the cell, which then inhibits the β-oxidation of fatty acids in the liver. Furthermore, ethanol metabolism also increases the rate of esterification of fatty acids.[12] The net effect is to favor fat accumulation in the hepatocytes. However, ethanol metabolism is not solely responsible for steatosis in the liver. Indeed, many pharmacological agents and genetic manipulations (e.g. knockouts) block steatosis in rodent models of alcohol administration. For example, mice deficient in pro-oxidant-producing enzymes (e.g. NADPH oxidase and inducible nitric oxide synthase)[13,14] or lipopolysaccharide (LPS) binding/signaling molecules (e.g. CD14, TLR4 and LBP), all have less steatosis in response to alcohol compared with wild-type mice.[15–17] However, these pharmacological/genetic alterations did not affect alcohol metabolism, suggesting that there is still the shift in the NADH ratio to a more reduced state.

A separate (but complementary) mechanism by which alcohol may cause fatty liver is via increasing the release of mediators that alter lipid metabolism; one example is the proinflammatory cytokine, tumor necrosis factor alpha (TNF-α).[18] This cytokine increases free fatty acid release from adipocytes in the periphery,[19] increases *de novo* lipid synthesis in hepatocytes[20] and inhibits β-oxidation of fatty acids.[21] Cytokines induced by alcohol may also impair transport and secretion of triglycerides such as very low-density lipoprotein (VLDL).[22] Therefore, TNF-α and other proinflammatory cytokines can increase the deposition of fatty acids in the liver, while at the same time block the ability of liver to properly break down and secrete these lipids. In support of the role of TNF-α in ethanol-induced fatty liver, TNFR1 knockout mice were protected against steatosis caused by alcohol.[23,24] Such a mechanism may explain the effect of the knockouts on steatosis mentioned above, as all did (or should) blunt TNF-α release and/or signaling.[13–17]

Alcoholic steatohepatitis

The next stage of ALD that may develop is steatohepatitis. For clarification, steatohepatitis refers to the pathological condition and not the clinical condition of alcoholic hepatitis, which is an often lethal complication of ALD.[25,26] This stage of ALD is considered to be a rate-limiting step in the formation of fibrosis/cirrhosis. Steatohepatitis is characterized histologically by both macro- and microvesicular steatosis, and infiltration of inflammatory cells, as well as hepatocyte degeneration, ballooning, necrosis and apoptosis.[27] Like simple steatosis, steatohepatitis is in principle also reversible with cessation of alcohol abuse. However, unlike steatosis, the reversion of steatohepatitis can take several weeks to months, instead of a matter of days.[28]

It is hypothesized that steatohepatitis involves an inappropriately activated immune/inflammatory response from stimuli that would normally be innocuous. First, inflammatory cells are 'primed' to be stimulated. For example, Deaciuc *et al*.[29] showed that rat livers tolerize to chronic low-dose LPS on exposure, but this tolerance is lost if alcohol is concomitantly administered. Monocytes isolated from patients with alcoholic hepatitis produce larger amounts of basal and stimulated TNF-α.[30] Not only does ethanol exposure increase the synthesis of TNF-α and other proinflammatory cytokines/chemokines (e.g. interleukin [IL]-6, IL-8, etc.), it also decreases the levels of anti-inflammatory cytokines (e.g. IL-10).[31–33] Second, steatosis 'sensitizes' the organ to a second insult, leading to more robust inflammatory liver damage.[11] For example, fatty livers are more susceptible to endotoxin- or cytokine-induced liver damage.[34,35] Ethanol exposure changes the effect of TNF-α on hepatocytes from a proproliferative to proapoptotic response, and liver cells that overexpress CYP2E1 become more sensitive to cell death owing to TNF-α after ethanol exposure.[34,36,37]

These two scenarios, priming and sensitization, could be considered a series of events that operate in tandem to cause the liver injury associated with ALD. Specifically, ethanol

primes inflammatory cells to release proinflammatory cytokines (e.g. TNF-α) that damage liver cells, which are sensitized to such damage. Therefore, a proper therapy for ethanol-induced liver inflammation may need to target both the priming and sensitization effects caused by ethanol exposure.

Fibrosis and cirrhosis

Fibrosis is characterized by deposition of extracellular matrices (ECMs). The main ECM to accumulate is collagen type I, but other ECM proteins also accumulate during fibrogenesis, including laminin, fibronectin and fibrin(ogen).[38] The major cell type that contributes to fibrogenesis is the hepatic stellate cell that has transdifferentiated into a myofibroblast-like phenotype. However, other cellular origins of myofibroblast-like cells are becoming increasingly recognized, such as periportal fibroblasts, fibrocytes and transdifferentiated epithelia.[39–42] This pathological change used to be considered irreversible. However, more recent studies in animal models of fibrosis demonstrated that even severe fibrotic changes may regress with time (see reference[43] for a review). It has also been shown that in humans, hepatitis C virus-induced liver fibrosis can be reversed if the underlying infection is effectively treated.[44] This exciting finding raises the prospect that advanced liver disease due to alcohol may also be reversible if the appropriate therapy can be identified. There are candidate strategies that have been identified in animal models that may show promise as emerging therapies, such as enhancing myofibroblast apoptosis,[45] blocking transforming growth factor β (TGF-β)/SMAD3 signaling,[46] enhancing matrix resolution (e.g. see reference[47]) and/or blocking inhibitors of matrix resolution.[48]

A basic limitation in alcoholic liver disease (ALD) research is that no rodent model completely recapitulates the human disease. Rodents appear more resistant to hepatic fibrosis caused by alcohol relative to humans. Indeed, with rare exceptions,[49] rodent alcohol models do not develop fibrotic changes. Thus, surrogate models of hepatic fibrosis (e.g. bile duct ligation [BDL] and carbon tetrachloride [CCl_4]) are predominantly used.[50] Whereas these models have many similarities to the human disease, there are also differences, as well as differences between the models.[50] A weight-of-evidence approach using multiple models is therefore most appropriate to identify new mechanisms or therapeutic targets.[51]

If the insult(s) responsible for liver damage persist past fibrosis, cirrhosis can develop. Cirrhosis consists of hepatic scarring similar to fibrosis but the scarring is more extensive. Cirrhosis is further characterized by altered liver parenchyma with septae and nodule formation, as well as distorted hepatic blood flow.[52,53] Upon the development of cirrhosis, death will most likely occur in the absence of a liver transplant.[53]

PAI-1 and its potential role in ALD initiation and progression

PAI-1 is an acute phase protein that is usually only expressed in adipocytes and endothelial cells, but it can be highly expressed by most cells in response to stress.[54] The classical role of PAI-1 is to inhibit the plasminogen activators, tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) (see Figure 1). Via attenuating the activation of plasminogen to plasmin, PAI-1 plays a major role in fibrin metabolism by blocking fibrinolysis. The role of PAI-1 in fibrin accumulation in vascular disease is well understood to contribute to endothelial dysfunction and inflammation. Although the liver can produce large amounts of PAI-1 in response to stress, the role of PAI-1 in liver diseases is not well understood.[55] Increased fibrinolysis is commonly observed in a cirrhotic liver and has been shown to be predictive of hepatic disease severity and the outcome of an inflicted patient.[56,57] However, although PAI-1 activity is known to be downregulated during cirrhosis, this protein is actually induced by alcohol administration[58–60] and levels of PAI-1 are a measure of liver disease severity during earlier stages of the disease.[61] However, whether the induction of PAI-1 mRNA levels is a cause or an effect of liver disease is not completely understood.

PAI-1 in alcohol-induced steatosis

As mentioned above, TNF-α and other proinflammatory cytokines potentially play major roles in causing steatosis after alcohol exposure; one mechanism by which these cytokines could mediate this effect is via inducing PAI-1 expression.[62] Work by this group has shown that both acute and chronic ethanol exposure robustly induce PAI-1 expression in the mouse liver. Furthermore, acute and chronic alcohol-induced steatosis was prevented by genetic or pharmacological inhibition of PAI-1 expression.[63] Similar protection against ethanol-induced steatosis was observed in TNFR1$^{-/-}$ mice in that study, a strain in which the increase in PAI-1 expression caused by ethanol was also completely blunted. Taken together, these data suggest that PAI-1 plays a critical role in steatosis caused by

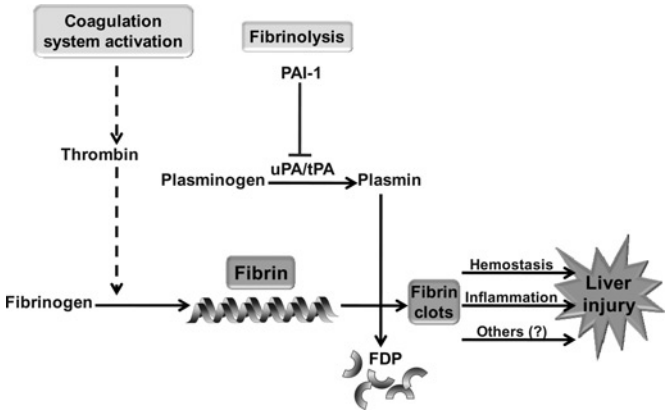

Figure 1 Schematic representation of the role of plasminogen activator inhibitor-1 (PAI-1) in fibrin(ogen) metabolism *in vivo*. Cross-linked fibrin deposition is initiated by activation of the coagulation cascade via thrombin. PAI-1 inhibits the activity of the plasminogen activators (uPA and tPA), blocking the activation of plasminogen to plasmin and thereby blunting degradation of fibrin matrices (fibrinolysis). The balance between fibrin deposition and degradation determines whether fibrin accumulates *in vivo*. uPA, urokinase plasminogen activator; tPA, tissue plasminogen activator; FDP, fibrin degradation product

acute and chronic alcohol exposure, analogous to previous findings in experimental non-alcoholic fatty liver disease.[64]

Although the most recognized function of the plasminogen activator system is to regulate fibrinolytic activity, proteolytic cleavage by plasminogen activators also modulates the activities of other proteins. For example, plasminogen activators cleave latent hepatocyte growth factor (HGF) to the mature active form,[65,66] which mediates lipid metabolism in hepatocytes by stimulating lipoprotein secretion.[67] Chronic ethanol treatment is known to downregulate expression (and signaling) of the HGF receptor (c-Met) and thereby block lipoprotein secretion.[68] Based on these findings, it was hypothesized that PAI-1, via inhibition of uPA/tPA, prevents the maturation of latent HGF, thereby impairing the HGF-dependent pathway of VLDL synthesis. In support of that hypothesis, genetic or pharmacological inhibition of PAI-1 expression caused an increase in c-Met phosphorylation and apolipoprotein B expression, as well as VLDL secretion after alcohol exposure.[63] These data suggest that the protective effect under these conditions is due to a compensatory increase in VLDL synthesis after ethanol exposure, which is blocked indirectly by PAI-1.

PAI-1 and hepatic inflammation

As mentioned above, steatosis and inflammation are linked in the progression of ALD. Indeed, in addition to blocking steatosis in the liver, preventing PAI-1 induction completely protected against chronic alcohol-induced inflammation.[63] However, the protective effect against inflammation was more robust than against steatosis. These results suggest that PAI-1 confers proinflammatory effects independent of steatosis in liver. Indeed, the enhanced LPS-induced inflammatory liver injury caused by ethanol pre-exposure was shown to be mediated, at least in part, by fibrin accumulation in livers, mediated by an inhibition of fibrinolysis by PAI-1.[69] These results are parallel to work in a mouse model of glomerulonephritis;[70] specifically PAI-$1^{-/-}$ mice had fewer infiltrating leukocytes and less severe damage to the glomeruli. A similar anti-inflammatory effect of knocking-out PAI-1 was observed during the early (inflammatory) phase of the BDL model ([71] see below).

Although PAI-1 is well known to be induced during inflammation, how PAI-1 may actually mediate inflammation is less understood; previous studies suggest potential mechanisms (Figure 2). First, the 'classical' role of PAI-1 in impairing fibrinolysis may also contribute to inflammation. For example, fibrin matrices have been shown to be permissive to chemotaxis and activation of monocytes and leukocytes.[72,73] Furthermore, fibrin clots disrupt the flow of blood within the hepatic parenchyma (i.e. hemostasis); the subsequent microregional hypoxia and hepatocellular death may directly (e.g. via HIF1α) and indirectly alter inflammatory cell signaling.[74,75]

Fibrin(ogen) ECM not only serves as physical structure but also binds/interacts with several biomolecules that can directly or indirectly alter responses (Figure 2). One family of receptors for which fibrin(ogen) is a ligand are the integrins. Integrins are receptors that mediate attachment

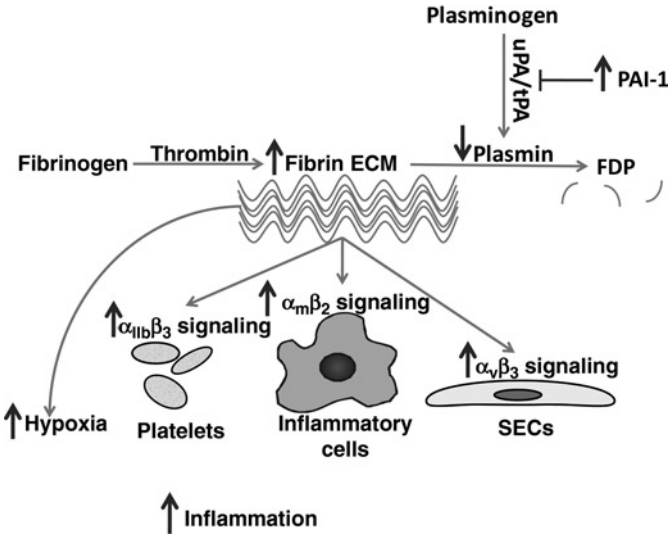

Figure 2 Potential mechanisms by which fibrin(ogen) contributes to hepatic inflammation caused by ethanol. Chronic ethanol increases hepatic fibrin extracellular matrix (ECM) deposition. This effect could contribute to alcohol-induced inflammation via one or more of the following mechanisms: (1) slowing blood flow in the liver (hemostasis), thereby increasing hypoxia; (2) stimulating platelets via integrin $\alpha_{IIb}\beta_3$ activation; (3) stimulating inflammatory cells via integrin $\alpha_M\beta_2$ activation; and (4) directly stimulating endothelial cells via integrin $\alpha_v\beta_3$ activation. uPA, urokinase plasminogen activator; tPA, tissue plasminogen activator; FDP, fibrin degradation product; PAI-1, plasminogen activator inhibitor-1; SECs, stimulated endothelial cells

between a cell and the tissues surrounding it, which may be other cells or ECM. Integrins transfer information from the ECM to the cell, allowing rapid and flexible responses to changes in the environment. Integrins play a myriad of roles within the body, including proliferation/angiogenesis, as well as inflammation and apoptosis.[76,77] Fibrin(ogen) is a known ligand for several integrins, including integrin $\alpha_{IIb}\beta_3$, and integrin $\alpha_M\beta_2$ and integrin $\alpha_v\beta_3$. These integrins are found on several non-parenchymal cells in the liver. Therefore, fibrin(ogen) ECM has the potential to alter inflammatory signaling in the liver via a myriad of mechanisms. Modulators of integrin function are in human clinical trials for non-hepatic diseases, and may ultimately be used in hepatic problems such as ALD.

In addition to altering fibrin metabolism, PAI-1 may alter the profile of other inflammatory mediators via inhibition of plasminogen activators. For example, the inhibition of plasmin activation by PAI-1 prevents the conversion of secreted latent TGF-β to its active form,[78] which may mediate anti-inflammatory effects, especially on monocytes/macrophages.[79] Furthermore, PAI-1 stabilizes other proteins by inhibiting plasmin formation, such as the chemotractant IL-8.[80] Furthermore, these mechanisms are not mutually exclusive and may occur in tandem. Indeed, previous studies have indicated that neutrophils contribute significantly to the induction of PAI-1 in a model of idiosyncratic drug toxicity that involves a significant inflammatory component.[81] Similar effects of PAI-1 were found in a model of severe hepatic inflammation/failure caused by partial hepatectomy coupled with low dose LPS.[82] Current preliminary studies by this group indicate that similar mechanisms contribute to inflammation after PAI-1 induction in experimental ALD.

PAI-1 and hepatic fibrosis

In addition to regulating the accumulation of fibrinogen/fibrin in the extracellular space, PAI-1 can modulate the metabolism of other ECM proteins via inhibiting the plasminogen activating system (see Figure 3). For example, in addition to fibrin, plasmin also directly degrades other ECM components such as laminin, proteoglycan and type IV collagen.[83–85] Furthermore, plasmin can also indirectly degrade ECM by activating matrix metalloproteinases (MMPs).[86] Thus, by impairing the plasminogen activating systems, PAI-1 alters organ fibrogenesis. Indeed, a protective effect of pharmacologic/genetic prevention of PAI-1 induction has been observed in models of renal, pulmonary and vascular remodeling, which share mechanisms with hepatic fibrosis.[87–89]

Based on studies with isolated and cultured stellate cells, it was proposed that PAI-1 may be both antifibrotic and profibrotic in liver, the former being mediated by the inhibition of interstitial collagenases during early stages of fibrosis.[90] However, a specific role of PAI-1 in hepatic fibrogenesis *in vivo* had not been experimentally determined. For reasons that remain unclear, rodent strains are highly resistant to developing hepatic fibrosis with alcohol exposure. Alternative models to study hepatic fibrogenesis are therefore used. The hypothesis that PAI-1 contributes to hepatic fibrosis was accordingly tested in the BDL model by comparing liver injury and fibrosis in wild-type and PAI-$1^{-/-}$ mice.[71] It was shown in that study that PAI-1 was induced by BDL in mice as early as three days after surgery. In addition to protecting against early inflammatory changes in the model, PAI-$1^{-/-}$ mice were dramatically protected against ECM accumulation, as determined by histological and biochemical assessments. Later work by others confirmed these findings.[91]

There are multiple levels at which hepatic fibrosis is regulated.[92] A major source of regulation is the transformation of stellate cells to myofibroblasts and production of ECM by these cells. Interestingly, increases in indices of this process (alpha smooth muscle actin and collagen Iα1 mRNA expression) were not attenuated in PAI-$1^{-/-}$ mice, despite preventing ECM accumulation.[71] It therefore appears unlikely that knocking out PAI-1 confers protection against hepatic fibrosis caused by BDL via blocking ECM synthesis. In addition to regulating synthesis of ECM, hepatic fibrogenesis is also determined by the balance between enzymes that degrade ECM (e.g. MMPs) and their inhibitors (e.g. tissue inhibitors of metalloproteinases [TIMPs]).[93] When indices of these processes were determined, it was shown that MMP-2 and -9 activities were elevated in PAI-$1^{-/-}$ mice, compared with wild-type mice.[71] This elevated collagenase activity was not associated with a decrease in amount of inhibitors (TIMPs) in PAI-$1^{-/-}$ mice, but rather an increase in plasminogen activator (tPA and uPA) activities, which indirectly activate MMPs via plasmin (see Figure 3).

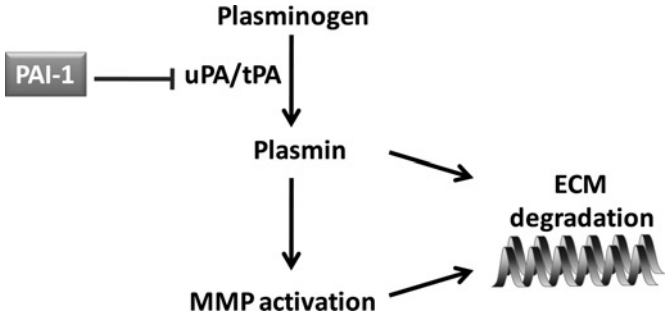

Figure 3 Proposed mechanisms by which plasminogen activator inhibitor-1 (PAI-1) inhibition protects against hepatic fibrosis. PAI-1 is a potent inhibitor of tPA and uPA, which convert plasminogen to plasmin. Plasmin can directly degrade extracellular matrix (ECM) components (e.g. fibrin, fibronectin, laminin, proteoglycan and type IV collagen). Plasmin can also indirectly degrade ECM via activation of MMPs (e.g. MMP-3[71]). uPA, urokinase plasminogen activator; tPA, tissue plasminogen activator; MMP, matrix metalloproteinase

Recent studies have identified a beneficial role of inhibiting TIMPs in CCl_4-induced fibrosis in rats.[94] The results of studies with PAI-$1^{-/-}$ mice in the BDL model suggest that agents that target the induction or activity of PAI-1 may also be beneficial and/or complementary to drugs that target TIMP activation. However, most studies in humans thus far have focused on the role of the plasminogen system on the development of the hyperfibrinolytic state with advanced liver cirrhosis (e.g.[95]). Whether or not PAI-1 contributes to the initiation and progression of fibrosis in humans should be investigated.

No rodent model completely recapitulates the fibrogenesis observed in chronic fatty liver diseases in humans. Thus, weight-of-evidence using multiple models of hepatic fibrosis is often used to identify new mechanisms. Therefore, although the results with BDL support the hypothesis that PAI-1 contributes to hepatic fibrosis in that model, and PAI-1 has been shown to be induced in other animal models of hepatic fibrosis,[96] results with this model alone were insufficient. An additional model often employed is fibrosis caused by CCl_4. In addition to differences in the pattern of fibrosis between BDL and toxin-induced (e.g. CCl_4) fibrosis, hepatocyte death and inflammation are generally more robust in the toxin-induced fibrosis models compared with BDL.[51,97] Thus positive results in both models would lend strong weight to the hypothesis that PAI-1 is involved in hepatic fibrosis in humans.

Previous studies investigating the plasminogen system indirectly supported such a possible function in the CCl_4 model. For example, genetic deletion of plasminogen (functionally analogous to high PAI-1 levels) exacerbated hepatic fibrogenesis in response to CCl_4.[98] Furthermore, increasing the conversion of plasminogen to plasmin by adenoviral overexpression of uPA in rat liver has been shown to accelerate the recovery from CCl_4-induced liver fibrosis.[99] It was therefore assumed *a priori* that knocking out PAI-1 would confer protection against CCl_4-induced fibrosis. However, surprisingly in contrast to findings in the BDL model,[71] liver damage and fibrosis were dramatically enhanced in PAI-1 knockout mice after chronic CCl_4 exposure.[100] The enhanced fibrosis after chronic CCl_4 in PAI-$1^{-/-}$ mice could be explained, in part, by increases in fibrogenesis and decreases in ECM degradation in this strain under these conditions. However, there were other effects in the knockout strain that are less likely to be explained by the above-mentioned changes. For example, liver damage was

robustly elevated, and compensatory hepatocyte proliferation was dramatically impaired.[100] These data suggest that the increased liver damage observed after chronic exposure to CCl_4 is likely secondary to an impaired proliferative response. A recent study has also observed a similar apparent proproliferative role of PAI-1 in mouse liver after experimental acetaminophen toxicity,[101] which is in line with these findings. It is hypothesized that this impaired ability of the PAI-1 knockout liver to restitute itself enhances the amount of liver damage and accelerates the fibrotic process during chronic CCl_4 exposure.

Given that previous studies have shown a profibrotic effect of PAI-1 in the BDL model,[71] the results in the CCl_4 model[100] were somewhat surprising. Furthermore, the apparent proproliferative role of PAI-1 under these conditions is also surprising. It is known that high PAI-1 levels correlate with poor prognosis for several cancers, and that PAI-1 levels correlate with cell growth and tumor aggressiveness *in vitro* and in animal models.[102] Therefore, further elucidation of the mechanisms by which PAI-1 mediates the effects observed here may shed light on the 'PAI-1 paradox' in cancer research.

As already mentioned, it is sometimes difficult to extrapolate from models of fibrosis to human disease, especially under conditions where the results in different models are contradictory, as has been observed for PAI-1 in the BDL and CCl_4 models. However, the relative differences in the two models may yield some information. Specifically, liver damage in the BDL model is relatively moderate (i.e. transaminases < 1000 IU/L). In contrast, CCl_4 causes massive hepatocyte damage. It is therefore likely that the damaging role of PAI-1 observed experimentally (e.g.[71]) better represents the role of PAI-1 in chronic liver diseases in humans with less robust proliferation after injury (e.g. ALD). However, if liver injury is severe (e.g. after acetaminophen,[101] liver failure and in liver transplant), the beneficial role of PAI-1 may be unmasked.

Recent clinical and basic studies indicate that the renin–angiotensin system is involved in hepatic fibrosis.[103] In attempts to address the apparent contradiction between the BDL and CCl_4 models, the role of PAI-1 in a new mouse fibrosis model caused by angiotensin II (AngII) infusion was determined.[104] AngII caused mild perisinusoidal fibrosis in wild-type mice, more similar to early stages in humans, without causing any significant inflammation and hepatocellular death. These results are in contrast with most other animal models of fibrosis that show at least a detectable increase in hepatic inflammation.[105,106] However, whether inflammation is required for fibrosis remains unclear, as some fibrotic diseases (e.g. hemochromatosis) do not possess a significant inflammatory component.

De novo synthesis is not the sole mechanism by which collagen ECM accumulation is regulated.[107] Another point of regulation is post-translational modification by prolyl-4-hydroxylases; this step is critical for the stability of the collagen ECM triple helix.[108] In that study,[104] AngII infusion significantly increased the expression of P4Hα, a key subunit for prolyl-4-hydroxylase, and also increased hydroxyproline content, supporting the hypothesis that AngII increased collagen deposition under these conditions, not via *de novo* synthesis, but rather by increasing proline hydroxylation.[104] This is in line with published studies in other organs.[109] Knocking out PAI-1 significantly increased hepatic MMP9 and MMP2 activities after AngII infusion, suggesting that blocking PAI-1 favored ECM degradation after AngII infusion.[104] These results are in line with the findings in the BDL model of hepatic fibrosis.[71]

Summary and conclusions

A safe and effective therapy for ALD in humans is still elusive, despite significant advances in our understanding of how the disease is initiated and progresses. PAI-1 may play a critical role in all stages of the natural history of ALD (steatosis, inflammation and possibly fibrosis). Therefore, targeting PAI-1 expression could confer beneficial effects not only in early alcohol-induced liver damage, but also in later stages of the disease (i.e. fibrosis and cirrhosis). Whereas potential mechanisms by which PAI-1 mediates steatosis (impaired VLDL synthesis) and fibrosis (enhanced ECM degradation) have been identified,[63,71] the mechanisms by which PAI-1 increases hepatic inflammation still needs to be more clearly elucidated. The mechanism by which PAI-1 is induced in chronic liver diseases is unclear. Indeed, PAI-1 expression is responsive to a number of different inducers (e.g. cytokines, AngII, hypoxia and oxidative stress[110–113]), many of which are suspected to be involved in alcohol-induced liver disease. Future studies should identify the cause(s) of elevated PAI-1 in ALD as a possible therapeutic target.

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