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New role of plasminogen activator inhibitor-1 in alcohol-induced liver injury

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Abstract

Plasminogen activator inhibitor-1 (PAI-1) is the main inhibitor of plasminogen activators, thereby playing a major role in fibrinolysis. Whereas hyperfibrinolysis is common in alcoholic cirrhosis, hypofibrinolysis (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 ALD has been unclear. The role of PAI-1 was therefore investigated in models of early (steatosis), intermediate (inflammation/necrosis) and late (fibrosis) stages of alcoholic liver disease. For example, hepatic steatosis caused by both acute and chronic ethanol was blunted by inhibiting PAI-1 activation. This effect of inhibiting PAI-1 appears to be mediated, at least in part, by an increase in very low-density lipoprotein (VLDL) synthesis in the absence of PAI-1. The results from that study also indicated that PAI-1 plays a critical role in both acute and chronic hepatic inflammation. Lastly, knocking out PAI-1 potently protected against experimental hepatic fibrosis; the mechanism of this protective effect appears to be mediated predominantly by extracellular matrix (ECM) resolution by matrix metalloproteases, which are indirectly inhibited by PAI-1. In summary, targeting PAI-1 protects against all three stages of ALD in model systems. The mechanisms by which PAI-1 contributes to these disease stages appear to not only involve the ‘classical’ function of PAI-1 (i.e. in mediating fibrinolysis), but also other functions of this protein. These data support a role of PAI-1 in the initiation and progression of ALD, and suggest that PAI-1 may be a useful target for clinical therapy to halt or blunt disease progression.

Keywords

cirrhosis; fatty liver; fibrosis; hepatitis; plasminogen activating system

Introduction

Alcoholic liver disease (ALD) affects millions of patients world-wide each year. The economic burden from alcoholism on the US economy, in part due to health care for individuals with ALD, rose 42% to USD \$148 billion from 1985 to 1992.¹ The disease process is characterized by early steatosis, inflammation and necrosis (often called steatohepatitis), and in some individuals ultimately progresses to fibrosis and cirrhosis. Although the progression of ALD is well characterized, there is no universally accepted therapy available to halt or reverse this process in humans. Instead, clinical treatment focuses predominantly on reducing the effects of decompensation caused by the disease and transplantation of livers of individuals with terminal cirrhosis.² Furthermore, whereas the risk of ALD increases in a dose- and time-

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Conflict of interest

No conflict of interest has been declared by the author.

dependent manner with consumption of alcohol,³⁻⁵ only a minor proportion of even heavy drinkers develop the severe form of the disease, suggesting that other environmental (e.g. hepatitis B virus [HBV] or hepatitis C virus [HCV] infection) or genetic (e.g. gender or polymorphisms in key genes) factors contribute to overall risk.⁶ With better understanding of the mechanism(s) and risk factors that mediate the initiation and progression of ALD, rational targeted therapy can be developed to treat or prevent ALD in the clinical setting. The present review summarizes recent findings with plasminogen activator inhibitor-1 (PAI-1) in experimental models of early (steatosis), intermediate (inflammation and necrosis) and late (fibrosis/cirrhosis) ALD.

PAI-1 is normally only expressed in adipocytes and endothelial cells; however, this acute-phase protein can be induced to high levels under conditions of injury and/or inflammation.⁷ PAI-1 is a major inhibitor of both tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). It therefore plays a major regulatory role in fibrinolysis by inhibiting the activation of plasminogen (Fig. 1). Owing to its critical role in blunting fibrinolysis, therapies targeted against PAI-1 are of major interest in preventing vascular diseases.⁸ However, the potential role of PAI-1 in liver diseases is less clear. Hyperfibrinolysis (high PA/PAI-1 ratio) is common in cirrhosis, and has been shown to be predictive of disease severity and patient outcome.^{9,10} In contrast to end-stage liver disease, PAI-1 is known to be upregulated with alcohol consumption¹¹⁻¹³ and its level is an index of the severity of the disease.¹⁴ However, whether elevated PAI-1 expression is a cause or an effect in the development of liver disease remains unclear.

PAI-1 in alcohol-induced steatosis

One of the earliest hepatic changes caused by alcohol is steatosis. Whereas originally thought to be a pathologically inert histological change, more recent work indicates that steatosis may play a critical role not only in the initiation, but also in the progression of ALD.^{15,16} For example, fatty livers are more sensitive to hepatotoxicity caused by agents such as endotoxin.¹⁵ Furthermore, the degree of fatty infiltration is predictive of the severity of later stages of ALD (i.e. fibrosis and cirrhosis).^{17,18}

Steatosis owing to alcohol has historically been considered the direct result of alcohol metabolism. Specifically, the metabolism of alcohol causes a shift in the cellular NADH pools to a more reduced state. The net effect of this redox shift is that fatty acid synthesis and esterification is increased with a simultaneous decrease in mitochondrial β -oxidation of fatty acids, causing lipids to accumulate in the hepatocyte. However, whereas alcohol metabolism is indeed likely to contribute to alcohol-induced steatosis, this process alone may not be sufficient to explain alcohol-induced steatosis. For example, many pharmacological agents have blunted experimental alcohol-induced steatosis without any apparent effect on alcohol metabolism, per se.¹⁹⁻²¹ Furthermore, many knock-out mouse strains (e.g. NADPH oxidase, inducible nitric oxide synthase [iNOS] and CD14)²²⁻²⁴ are protected against experimental alcohol-induced steatosis without altering alcohol metabolism. These results therefore suggest that alcohol metabolism is not the sole cause of alcoholic fatty liver.

An additional pathway by which ethanol may cause steatosis is via inducing cytokine (e.g. tumor necrosis factor [TNF- α]) production.²⁵ For example, TNF- α increases free fatty acid release from adipocytes in the periphery,²⁶ increases lipogenesis in hepatocytes,²⁷ and inhibits β -oxidation of fatty acids.²⁸ Cytokines induced by alcohol may also impair transport and secretion of triglycerides as very low-density lipoprotein (VLDL).²⁹ Indeed, knocking out TNF- α receptor 1 (TNFR1) almost completely blunts alcohol-induced fatty liver.^{30,31} The net consequence during alcohol exposure is that cytokines theoretically increase the supply of fatty acids to liver while simultaneously impairing the ability of the hepatocytes to metabolize and

secrete them. However, the specific mechanism(s) by which cytokines may mediate these effects have not been completely determined.

One mechanism by which TNF- α could cause hepatic steatosis is via inducing PAI-1 expression. TNF- α is a known potent inducer of PAI-1 expression most likely via the mitogen-activated protein (MAP) kinases pathway.³² Work by our group has shown that acute ethanol rapidly and robustly induced PAI-1 expression in the mouse liver, and that steatosis under these conditions was prevented by genetic (PAI-1^{-/-} mice) or pharmacologic inhibition of PAI-1 expression.³³ Similar protection against ethanol-induced steatosis was observed in TNFR1^{-/-} mice in that study, a strain in which the induction of PAI-1 expression caused by ethanol was also significantly blunted. Steatosis owing to chronic enteral alcohol exposure was also blunted by preventing the induction of PAI-1 expression (Fig. 2).³³ Taken together, these data indicated that PAI-1 plays a critical role in alcohol-induced steatosis, analogous to previous findings in experimental non-alcoholic fatty liver disease (NAFLD).³⁴

As mentioned above, the most recognized function of the plasminogen activator system is to regulate fibrinolytic activity. However, 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.^{35,36} Results of *in vitro* studies suggested that activation of c-Met by HGF stimulates lipoprotein secretion in hepatocytes.³⁷ Furthermore, chronic ethanol treatment downregulates hepatic c-Met and apolipoprotein expression.³⁸ Based on these findings, it was hypothesized that PAI-1, via inhibition of urokinase-type plasminogen activator (uPA)/tissue-type plasminogen activator (tPA), prevents the maturation of latent HGF, thereby impairing the HGF-dependent pathway of VLDL synthesis (Fig. 3). In support of that hypothesis, genetic or pharmacologic inhibition of PAI-1 expression caused an increase in c-Met phosphorylation, apolipoprotein B expression, as well as VLDL excretion after alcohol exposure.³³ These data suggest that the protective effect under these conditions represents a compensatory increase in VLDL synthesis to account for the greater amount of lipids in the hepatocyte after ethanol exposure.

PAI-1 and hepatic inflammation

An interesting finding in the experiments with PAI-1^{-/-} mice exposed to chronic enteral ethanol is that, in addition to blunting steatosis, genetic inhibition of PAI-1 expression also conferred profound anti-inflammatory effects.³³ Indeed, whereas knocking out PAI-1 partially blunted the steatotic changes caused by chronic ethanol, there was almost complete protection against the inflammatory changes caused by alcohol in this strain (Fig. 4). A similar anti-inflammatory effect of knocking-out PAI-1 was observed by others in a mouse model of glomerulonephritis;³⁹ 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 bile duct ligation model,⁴⁰ which is discussed in more detail below. These results suggest that independent of steatosis, PAI-1 may mediate inflammatory effects *in vivo*.

Whereas PAI-1 is well known to be induced during inflammation, how PAI-1 may actually contribute to inflammatory processes is less understood. However, previous studies suggest potential mechanisms. 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.^{41,42} 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 transforming growth factor- β (TGF- β) to its active form,⁴³ which may mediate anti-inflammatory effects, especially on monocytes/macrophages.⁴⁴

Furthermore, PAI-1 stabilizes other proteins by inhibiting plasmin formation, such as the chemoattractant IL-8.⁴⁵ 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.⁴⁶ Similar effects of PAI-1 were found by this group in a model of severe hepatic inflammation/failure caused by partial hepatectomy coupled with low-dose LPS.⁴⁷ 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

As mentioned above, the final step in the natural progression of alcoholic liver disease (and other chronic liver diseases) is the accumulation of extracellular matrices (ECM), leading to fibrosis and possible progression to cirrhosis. In the absence of liver transplantation, the sequelae of end-stage liver disease will most often lead to the death of the patient.⁴⁸ It was recently shown in humans that liver fibrosis/cirrhosis can at least partially resolve if the underlying cause is effectively treated (e.g. hepatitis virus C infection).⁴⁹ However, due in part to an incomplete understanding of the mechanisms underlying hepatic fibrosis, no mechanism-based therapy to halt the progression or enhance the rate of resolution of this disease has been approved by the FDA.

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. For example, in addition to fibrin, plasmin also directly degrades other ECM components such as laminin, proteoglycan, and type IV collagen.⁵⁰⁻⁵² Furthermore, plasmin can also indirectly degrade ECM via activation of matrix metalloprotease (MMP).⁵³ Thus, by impairing the plasminogen-activating systems, PAI-1 significantly 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' (fibrosis).⁵⁴⁻⁵⁶

In liver, a correlation between PAI-1 levels and protection against fibrosis has been observed.⁵⁷ 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.⁵⁸ 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 bile duct ligation model by comparing liver injury and fibrosis in wild-type and PAI-1^{-/-} mice.⁴⁰ It was shown in that study that PAI-1 was induced by bile duct ligation in mice as early as 3 days after surgery. In addition to protecting against early inflammatory changes in the model (see above), PAI-1^{-/-} mice were dramatically protected against ECM accumulation after 2 weeks of bile duct ligation, as determined by histological and biochemical assessments. Later work by others confirmed these findings.⁵⁹

The results with bile duct ligation 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.⁶⁰ However, in addition to differences in the pattern of fibrosis between bile duct ligation and toxin-induced (e.g. CCl₄) fibrosis, hepatocyte death and inflammation are generally more robust in the latter models compared to bile duct ligation.^{61,62} Thus, whether or not PAI-1 is broadly involved in hepatic fibrogenesis cannot currently be concluded. While a direct role of PAI-1 in toxin-induced hepatic fibrosis has not been determined, previous studies investigating the plasminogen system indirectly support such a possible function. For

example, genetic deletion of plasminogen has been shown to exacerbate hepatic fibrogenesis in response to CCl₄.⁶³ Furthermore, increasing the conversion of plasminogen to plasmin by adenoviral overexpression of uPA in rat liver has been shown to accelerate the recovery from CCl₄-induced liver fibrosis.⁶⁴

There are multiple levels at which hepatic fibrosis is regulated.⁶⁵ 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 [α SMA]) and collagen I α 1 mRNA expression) were not attenuated in PAI-1^{-/-} mice, despite preventing ECM accumulation.⁴⁰ It therefore appears unlikely that knocking out PAI-1 confers protection against hepatic fibrosis caused by bile duct ligation via regulation of the above-described processes. In addition to regulating synthesis of ECM, hepatic fibrogenesis is also determined by the balance between enzymes that degrade ECM (e.g. MMP) and their inhibitors (e.g. tissue inhibitor of metalloproteases [TIMP]).⁶⁶ When indices of these processes were determined, it was shown that MMP-2 and -9 activities were elevated in PAI-1^{-/-} mice, compared to wild-type mice.⁴⁰ This elevated collagenase activity was not associated with a decrease in the amount of inhibitors (TIMP) in PAI-1^{-/-} mice, but rather an increase in tPA and uPA, which indirectly activate MMP via plasmin (Fig. 4).

Recent studies have identified a beneficial role of inhibiting TIMP in CCl₄-induced fibrosis in rats.⁶⁷ The results of studies with PAI-1 in the bile duct ligation 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. Whether or not PAI-1 is specific to cholestasis-induced fibrosis or is broadly involved in hepatic fibrogenesis is the focus of future studies. Furthermore, most studies in humans thus far have focused on the role of the plasminogen system on the development of the hyper-fibrinolytic state with advanced liver cirrhosis.⁶⁸ Whether or not PAI-1 contributes to the initiation and progression of fibrosis in humans should be investigated.

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. The work summarized in this review indicates that PAI-1 may play a critical role in all stages of the natural history of ALD (steatosis, inflammation and fibrosis). Therefore, targeting PAI-1 expression could confer beneficial effect 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 under these conditions,^{33,40} the mechanisms by which PAI-1 increases hepatic inflammation still needs to be more clearly elucidated. Last, 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, angiotensin II, hypoxia and oxidative stress),⁶⁹⁻⁷² 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.

Biography

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Figure 1.

Schematic representation of the role of PAI-1 in fibrin(ogen) metabolism *in vivo*. Cross-linked fibrin deposition is initiated by activation of the coagulation cascade via thrombin. Plasminogen activator inhibitor-1 (PAI-1) inhibits the activity of the plasminogen activators (urokinase-type plasminogen activator [uPA] and tissue-type plasminogen activator [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*.

Figure 2.

Photomicrographs of livers following chronic ethanol treatment: role of plasminogen activator inhibitor-1 (PAI-1). Representative photomicrographs (original magnification, 100×) of livers from wild-type and PAI-1^{-/-} mice fed control or ethanol-containing diet for 4 weeks are shown.³³ Higher magnification (200×; lower panels), shows inflammation and necrosis in wild-type mice fed ethanol-containing diet.

Figure 3.

Working hypothesis on the mechanism by which plasminogen activator inhibitor-1 (PAI-1) causes hepatic steatosis. In addition to inhibiting fibrinolysis (see Fig. 1), inhibition of urokinase-type plasminogen activator (uPA) prevents the cleavage/maturation of pro-hepatocyte growth factor (HGF). Decreased HGF signaling via the c-Met/ERK pathway leads to a decrease in the expression of apolipoprotein B (Apo) and microsomal triglyceride transfer protein (MTTP) in the cell. The net effect is a decreased assembly and secretion of lipoproteins, resulting in an intracellular triglyceride accumulation. Knocking out PAI-1 or blocking its induction (with metformin) prevents this effect on very low-density lipoprotein (VLDL) production.

Figure 4.

Proposed mechanisms by which plasminogen activator inhibitor-1 (PAI-1) inhibition protects against hepatic fibrosis. PAI-1 is a potent inhibitor of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (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 matrix metalloprotease (MMP) (e.g. MMP-3).⁴⁰