FEBS Letters 581 (2007) 3098–3104

PAI-1 deficiency reduces liver fibrosis after bile duct ligation in mice through activation of tPA

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Received 19 March 2007; revised 8 May 2007; accepted 18 May 2007

Available online 29 May 2007

Edited by Veli-Pekka Lehto

Abstract Plasminogen activator inhibitor-1 (PAI-1) increases injury in several liver, lung and kidney disease models. The objective of this investigation was to assess the effect of PAI-1 deficiency on cholestatic liver fibrosis and determine PAI-1 influenced fibrogenic mechanisms. We found that *PAI-1*$^{-/-}$ mice had less fibrosis than wild type (WT) mice after bile duct ligation. This change correlated with increased tissue-type plasminogen activator (tPA) activity, and increased matrix metalloproteinase-9 (MMP-9), but not MMP-2 activity. Furthermore, there was increased activation of the tPA substrate hepatocyte growth factor (HGF), a known anti-fibrogenic protein. In contrast, there was no difference in hepatic urokinase plasminogen activator (uPA) or plasmin activities between *PAI-1*$^{-/-}$ and WT mice. There was also no difference in the level of transforming growth factor beta 1 (TGF-β1), stellate cell activation or collagen production between WT and *PAI-1*$^{-/-}$ animals. In conclusion, PAI-1 deficiency reduces hepatic fibrosis after bile duct obstruction mainly through the activation of tPA and HGF.

Keywords: Plasminogen activator inhibitor-1; Extrahepatic cholestasis; Liver fibrosis; Matrix metalloproteinase; Tissue-type plasminogen activator; Urokinase plasminogen activator; Hepatocyte growth factor

1. Introduction

Liver fibrosis is the final common pathway leading to liver failure after many different types of injury. The process from initial insult to cirrhosis is a complex progression of necrosis/apoptosis, cell proliferation, inflammation and extracellular matrix deposition. Each aspect is controlled by specific molecular mediators. Many of these mediators, including matrix metalloproteinases (MMPs), transforming growth factor-beta 1 (TGF-β1) and hepatocyte growth (HGF), are produced as inactive precursors requiring proteolytic cleavage for activation. Therefore, controlled protease activity is essential in cellular responses and extracellular matrix (ECM) remodeling [1,2].

The plasmin protease system, consisting of plasminogen activator inhibitor-1 (PAI-1), urokinase plasminogen activator (uPA), tissue-type plasminogen activator (tPA) and plasmin, is best understood for its role in blood fibrinolysis [3]. PAI-1 prevents the over-production of plasmin by blocking the activity of tPA and uPA, proteases that convert the inactive protease plasminogen to the active protease plasmin. PAI-1 is also important in tissue remodeling and cell migration associated with tissue repair [4]. For example, studies of PAI-1 overexpression in transgenic mice demonstrate that PAI-1 promotes fibrin deposition and organ fibrosis [5,6]. In contrast, PAI-1 deficiency in mice attenuates kidney and lung fibrosis [6,7].

It was recently reported that PAI-1 deficiency also reduces liver injury [2] and fibrogenesis [8] in early stages (3–14 days) after bile duct ligation (BDL). These studies demonstrate that PAI-1 expression was significantly elevated early after BDL and that PAI-1 increases neutrophil infiltration and reduces pro-HGF processing in mice. Furthermore, one of these studies demonstrated that PAI-1 deficient mice have less collagen accumulation in the liver 2 weeks after BDL than wild type (WT) animals [8]. Whether PAI-1 deficiency results in less fibrosis at later time points after BDL is unknown, and the mechanism of reduced fibrosis in *PAI-1*$^{-/-}$ mice after BDL remains poorly understood. For example, PAI-1 deficiency might be expected to increase the activity of plasminogen activators (tPA and uPA) and the conversion of plasminogen to plasmin. Since plasmin is a potent activator of transforming growth factor β1, a major pro-fibrogenic protein, it might also be predicted that PAI-1 deficient mice would have elevated levels of activated TGFβ1, but this has not yet been evaluated. Thus, mechanisms that influence fibrogenesis in WT and PAI-1 deficient mice need additional study.

To clarify the role of PAI-1 in hepatic fibrosis after biliary tract obstruction at times with more established fibrosis, we have now determined the extent of activation of tPA, uPA, plasmin and their substrates in mice 3 weeks after BDL. We also determined the degree of collagen accumulation in WT and *PAI-1*$^{-/-}$ mice at this time point. In agreement with the prior report [8], the current investigation demonstrates that he-

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Abbreviations: BDL, Bile duct ligation; HGF, hepatocyte growth factor; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor-1; qRT-PCR, quantitative real time reverse transcriptase polymerase chain reaction; TGF-β1, transforming growth factor-beta 1; tPA, tissue-type plasminogen activator; uPA, urokinase plasminogen activator; WT, wild type

0014-5793/$32.00
doi:10.1016/j.febslet.2007.05.049

patic fibrosis is reduced in *PAI-1*$^{-/-}$ mice 3 weeks after BDL and that tPA activation is increased after BDL in *PAI-1*$^{-/-}$ compared to WT mice. This tPA activation correlates with the activation of known tPA substrates MMP9 and HGF, proteins that should reduce hepatic fibrosis. However, in contrast to the study by Bergheim et al. we find that uPA and plasmin levels are equivalent in *PAI-1*$^{-/-}$ and WT mice after BDL. Supporting these data is the observation that uPA and plasmin substrates MMP2 and TGF-β1 are also equivalent in WT and *PAI-1*$^{-/-}$ mice after BDL. Thus, for the first time, we demonstrate a distinct role of tPA, instead of uPA, in PAI-1 associated liver fibrosis. These data suggest that PAI-1 is pro-fibrogenic primarily because it reduces tPA activation after BDL.

2. Materials and methods

2.1. Mouse model

Male 8–10 weeks old (22–26 g) WT (#0664) and *PAI-1*$^{-/-}$ C57BL/6J mice (#2507) from the Jackson Laboratory (Bar Harbor, ME) underwent BDL. The common bile duct was double ligated below the bifurcation, single ligated above the pancreas and transected between the ligatures under methoxyflurane (Schering-Plough Co., Union, NJ) anesthesia. Sham mice underwent similar laparotomy without BDL. Three to five mice of each genotype with sham surgery and 5–7 mice of each genotype with BDL at each time point were sacrificed to obtain blood and liver samples. The use and humane care of mice were approved by Washington University's Animal Care Committee.

2.2. Fibrosis analysis

Morphometric analysis of Masson's trichrome and Sirius red stained liver sections [9] was performed on digital images (40×) of entire liver sections using NIH imageJ 1.30 (NIH, Bethesda, MD). Fibrotic area % = [collagen area/(total area − vascular lumen area)] × 100%. Hydroxyproline content was measured colorimetrically [9,10] with comparison to L-hydroxyproline standards.

2.3. HGF and smooth muscle α-actin (α-SMA) immunoblot

HGF immunoblot and α-SMA immunoblot analysis was performed as described [2]. Primary antibodies: anti-human α-SMA (1:100, clone 1A4, Dako) [11], goat polyclonal anti-human α-HGF antibody (1:100, N-17, Santa Cruz) [12,13].

2.4. α-SMA immunohistochemistry

Activated stellate cell marker α-SMA antibody (1:500, clone 1A4, Dako) [9] was used with secondary biotinylated IgG and Tyramide amplification (NEN Life Science Products, Boston, MA). Morphometric analysis were performed as described above.

2.5. MMP-2, MMP-9, tPA, uPA and plasmin activity

Casein and plasminogen zymography for tPA, uPA and plasmin activities was performed as described [2]. For MMP activity, gelatin zymography was performed similarly except 10% Novex Zymogram gels containing 0.1% gelatin (Invitrogen, Carlsbad, CA) were used. Hepatic plasmin activity was also measured using a fluorogenic substrate as described [2].

2.6. TGF-β1 immunoassay (EIA)

Two hundred microgram liver homogenate protein was analyzed for TGF-β1 using a Quantikine TGF-β1 EIA kit (R&D Systems Inc., Minneapolis, MN). Latent TGF-β1 was activated in 40 μL samples with 1 N HCl (9 μL) and later neutralized with 1.2 N NaOH (6 μL).

2.7. Quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR)

mRNA isolated using Micro-FastTrack 2.0 mRNA kit (Invitrogen, Carlsbad, CA) was reverse transcribed (SMART™ PCR cDNA synthesis kit, Clontech Inc., Palo Alto, CA). qRT-PCR was performed in duplicate using SYBR green PCR Master mix (Applied Biosystems) and an iCycler iQ (Bio-Rad). Primers are in Table 1 and Ref. [2]. RNA content was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

2.8. Statistical analysis

Data were reported as mean ± S.E.M. Statistical analyses used Student *t* test or Mann–Whitney rank sum test. $P < 0.05$ was considered significant.

3. Results

3.1. PAI-1 mRNA level remains elevated after BDL

The final common pathway for many types of liver injury includes stellate cell activation and collagen accumulation, eventually resulting in cirrhosis. Although PAI-1 mRNA was previously reported to be elevated shortly after BDL [2,8], whether PAI-1 elevation persists is not yet known. To determine if PAI-1 mRNA levels remain elevated 3 weeks after BDL, qRT-PCR was performed and demonstrated a marked elevation in PAI-1 mRNA compared to sham operated mice (fold change: BDL 41.7 ± 11.0 versus sham surgery 1.1 ± 0.1, $P < 0.01$). This suggests that persistent PAI-1 elevation may contribute to long-term changes in the liver in the setting of extrahepatic cholestasis.

3.2. PAI-1 deficiency reduces hepatic fibrosis after BDL

An earlier report suggested that PAI-1 deficiency reduces hepatic fibrosis 2 weeks after BDL [8]. To confirm and extend this observation, morphometric analysis of collagen deposition as demonstrated by Sirius Red and Masson's trichrome was performed on liver section obtained 3 weeks after BDL in WT and *PAI-1*$^{-/-}$ mice (Fig. 1A–C). This analysis demonstrated 18–26% reductions in hepatic fibrosis after BDL ($P \leqslant 0.03$) in *PAI-1*$^{-/-}$ compared to WT mice, but comparable hepatic collagen content after sham surgery. The reduced

Table 1
Primers used for qRT-PCR

Gene symbol	UniGene name	Sequences[a]
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	f AACTTTGGCATTGTGGAAGG r GTCTTCTGGGTGGCAGTGAT
Col1-α1	Procollagen I alpha 1	f GAACAGGGTGTTCCTGGAGA r GGAAACCTCTCTCGCCTCTT
α-SMA	Smooth muscle actin, alpha 2	f ATGAAGCCCAGAGCAAGAGA r ATGTCGTCCCAGTTGGTGAT

[a] 5′to 3′ sequences; f = forward primer; r = reverse-strand primer.

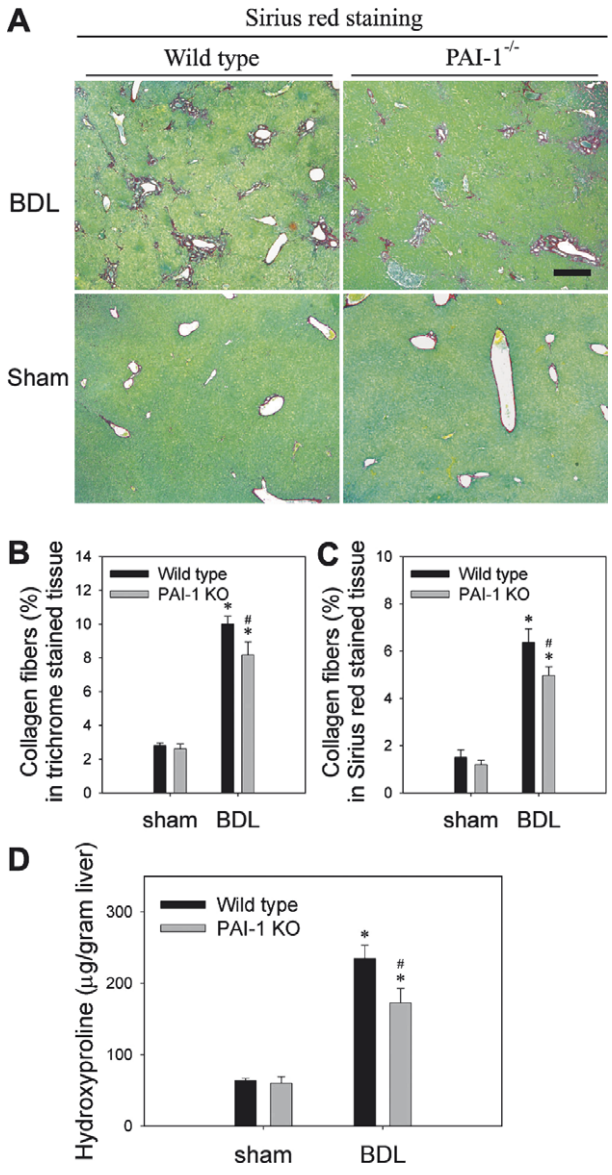

Fig. 1. Hepatic collagen content is lower in *PAI-1*$^{-/-}$ than in WT mice after BDL. (A) Representative Sirius red stained liver sections were obtained 3 weeks after BDL (40×, Scale bar = 300 μm). (B, C) Quantitative image analysis of collagen fibers with Masson's trichrome or Sirius red staining 3 weeks after BDL demonstrates that *PAI-1*$^{-/-}$ mice develop less fibrosis than WT animals. (D) Quantitative analysis of hepatic hydroxyproline content in liver tissue 3 weeks after BDL confirms reduced collagen content in *PAI-1*$^{-/-}$ compared to WT mice. Data represent means ± S.E.M. for 3–5 sham and 5–7 BDL mice. #$P \leqslant 0.03$ versus WT. *$P < 0.001$ versus sham of the same genotype.

collagen accumulation in *PAI-1*$^{-/-}$ mice after BDL was also confirmed by measuring hepatic hydroxyproline content (Fig. 1D). Thus, consistent with a previous report [8], PAI-1 deficiency attenuates liver fibrosis at later time points after BDL.

3.3. PAI-1 deficiency increases tPA activity after BDL, but not uPA or plasmin activity

PAI-1 inhibits tPA and uPA. Since tPA and uPA are plasminogen activators, it might also be expected that PAI-1 would inhibit plasminogen activation as it does in classically described fibrinolytic pathways [3]. Indeed, another group re-

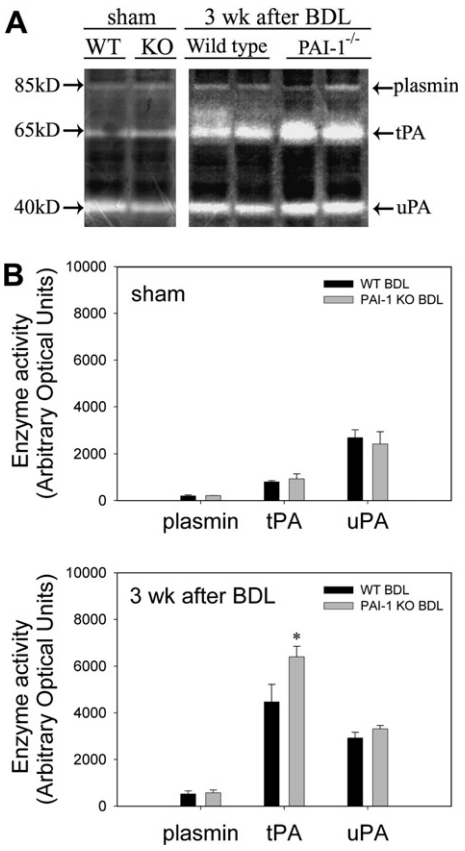

Fig. 2. Gel zymography analysis of tPA, uPA, and plasmin activity in WT and *PAI-1*$^{-/-}$ mice. (A) Casein/plasminogen zymography demonstrated the level of active uPA, tPA and plasmin in the liver at 3 weeks after BDL. (B) Quantitative analysis of casein/plasminogen zymography demonstrated increased tPA activity in the liver of *PAI-1*$^{-/-}$ versus WT animals after BDL, but no significant differences in uPA or plasmin activity after BDL and no difference in tPA, uPA or plasmin activity after sham surgery. Data represent means ± S.E.M. for 3–5 sham and 5–7 BDL mice. *$P \leqslant 0.02$ versus WT.

cently reported that both uPA and tPA activity were elevated in *PAI-1*$^{-/-}$ compared to WT mice after BDL [8]. In contrast, we had previously found that tPA activity was elevated in *PAI-1*$^{-/-}$ compared to *WT* mice, but uPA and plasmin activity were equivalent [2]. To determine if tPA and uPA are activated in a more established liver fibrosis model, we measured the activity of these proteins and their mRNA levels in *PAI-1*$^{-/-}$ and *WT* mouse liver 3 weeks after BDL using casein/plasminogen zymography and qRT-PCR, respectively. These studies demonstrated equivalent tPA and uPA mRNA levels in WT and *PAI-1*$^{-/-}$ mice after BDL. Furthermore, although uPA enzymatic activity was not statistically different between WT and *PAI-1*$^{-/-}$ mice, tPA enzymatic activity was 43% higher in *PAI-1*$^{-/-}$ than WT mice 3 weeks after BDL (Fig. 2, $P = 0.02$), but comparable in WT and *PAI-1*$^{-/-}$ after sham surgery. Together these data suggest that PAI-1 is an important regulator of tPA activity after cholestatic liver injury, but that uPA activity is primarily controlled by other mechanisms.

Because tPA activates plasminogen by proteolysis to plasmin, one might hypothesize that increased tPA activity in *PAI-1*$^{-/-}$ mice after BDL would cause higher plasmin activity. To test this hypothesis we measured hepatic plasmin activity

by casein gel zymography (Fig. 2), but found similar plasmin activity levels in WT and in *PAI-1*$^{-/-}$ mice 3 weeks after BDL. These findings were confirmed using a fluorogenic plasmin substrate assay (data not shown). Plasminogen mRNA levels were also equivalent in WT and *PAI-1*$^{-/-}$ mice after BDL. Together these data suggest that mechanisms other than PAI-1 control hepatic plasmin activity after BDL.

3.4. TGF-β1 activation, stellate cell activation and collagen synthesis are equivalent in *PAI-1*$^{-/-}$ and WT mice before and after BDL

The extent of fibrosis depends on rates of collagen synthesis and degradation. Because *PAI-1*$^{-/-}$ mice have reduced collagen accumulation after BDL, we were interested in determining whether rates of collagen production were also reduced. Stellate cell activation is an important mechanism of hepatic fibrogenesis and TGF-β1, a key stellate cell activator, is produced as an inactive precursor that can be activated by plasmin. Active and latent TGF-β1 levels were therefore measured in liver homogenates by enzyme immunoassay. Although active and latent TGF-β1 were elevated at 1 and 3 weeks after BDL compared to sham surgery (Fig. 3A), PAI-1 deficiency, did not influence the level of active or latent TGF-β1 in liver homogenates. Pro-collagen I alpha 1 mRNA levels were also comparable in WT and *PAI-1*$^{-/-}$ mice after BDL (Fig. 3B).

To evaluate stellate cell activation, we performed liver immunohistochemistry for the activated stellate cell marker α-SMA. While hepatic α-SMA^{+} cells increased dramatically after BDL, there was no difference between WT and *PAI-1*$^{-/-}$ mice (Fig. 4A and B). These results were confirmed by measuring α-SMA mRNA using qRT-PCR (Fig. 4C) and by protein immunoblot analysis (Fig. 4D). Collectively these data demonstrate that PAI-1 deficiency does not influence hepatic collagen accumulation by affecting collagen production or stellate cell activation.

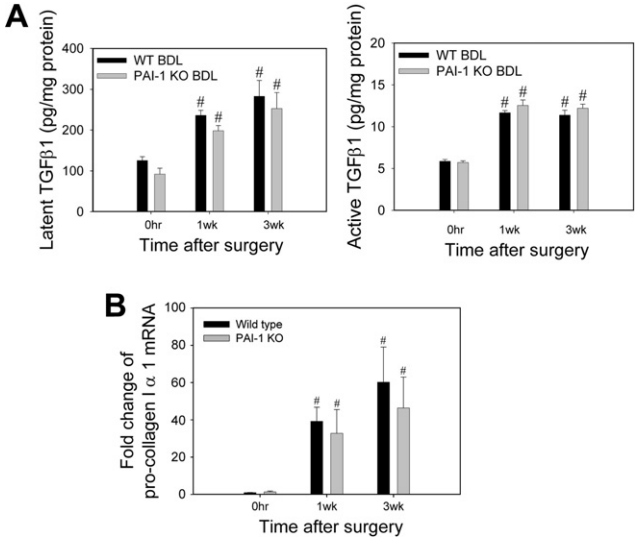

Fig. 3. TGF-β1 activation and pro-collagen I α1 mRNA production are equivalent in WT and *PAI-1*$^{-/-}$ mice after BDL. (A) Active and latent hepatic TGF-β1 levels as measured by EIA are equal in WT and *PAI-1*$^{-/-}$ mice at 1 and 3 weeks after BDL. (B) Pro-collagen I α 1 mRNA levels as evaluated by qRT-PCR are equal at 1 week and 3 weeks after BDL in WT and *PAI-1*$^{-/-}$ mice. Data represent means ± S.E.M. for 3–5 sham and 5–7 BDL mice. $^{\#}P < 0.01$ versus sham mice of the same genotype.

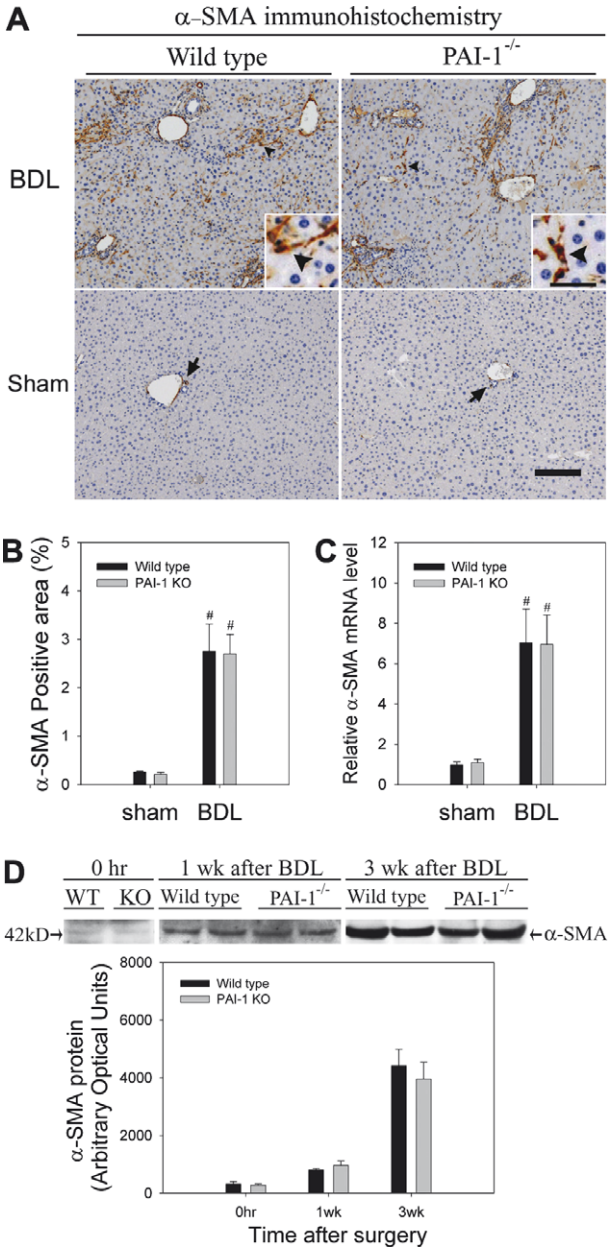

Fig. 4. Stellate cell activation is equivalent in WT and *PAI-1*$^{-/-}$ mice after BDL. (A) Stellate cell activation 3 weeks after BDL was evaluated by α-SMA immunohistochemistry (100×, scale bar = 150 μm) and cell morphology. Arrows show α-SMA expression in blood vessels. Inserts with arrow head show the morphology of activated stellate cells (scale bar = 50 μm). (B) Quantitative image analysis of α-SMA positive area 3 weeks after BDL demonstrates that *PAI-1*$^{-/-}$ and WT mice have equivalent amount of stellate cell activation. (C) Quantitative real-time PCR demonstrates that mRNA expression of α-SMA in liver 3 weeks after BDL is equivalent in *PAI-1*$^{-/-}$ and WT mice. (D) Hepatic α-SMA levels determined by protein immunoblot analysis are equivalent in WT and *PAI-1*$^{-/-}$ mice at 1 week and 3 weeks after BDL. Data represent means ± S.E.M. for 3–5 sham and 5–7 BDL mice. $^{\#}P < 0.01$ versus sham mice of the same genotype.

3.5. MMP9 levels are higher in PAI-1$^{-/-}$ than WT mice after BDL

Because PAI-1 deficiency does not alter collagen production after BDL, we hypothesized that reduced fibrosis in *PAI-1*$^{-/-}$ mice was due to increased collagen degradation. Since tPA increases MMP-9 synthesis [14] while uPA increases both MMP-2 and MMP-9 activity [15,16], we measured MMP-2 and MMP-9 activity in liver homogenates 3 weeks after BDL using gelatin zymography. Both pro-MMP-9 and MMP-9 were significantly elevated in *PAI-1*$^{-/-}$ compared to WT liver (Fig. 5). In contrast, pro-MMP2 and MMP2 levels were comparable in WT and *PAI-1*$^{-/-}$ mice. Collectively, these data suggest that reduced collagen accumulation in *PAI-1*$^{-/-}$ mice after BDL results from increased collagen degradation rather than reduced collagen synthesis.

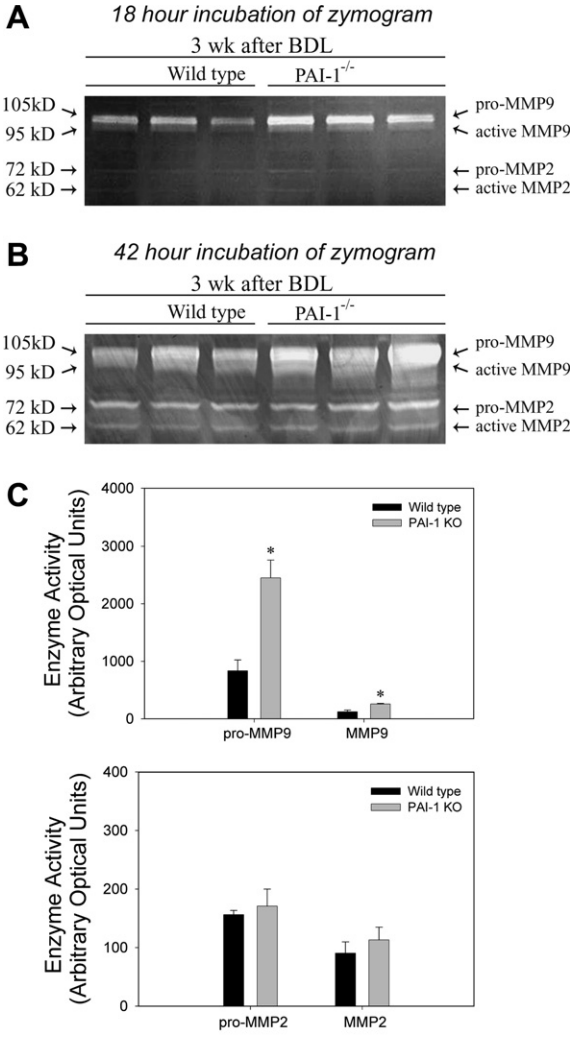

Fig. 5. Gel zymography analysis of MMP-9 and MMP-2 activity in WT and *PAI-1*$^{-/-}$mice. (A, B) Gelatin zymography demonstrated both active and pro-MMP proteins (MMP-2 and MMP-9) in the liver after BDL. (A) Zymogram was incubated 18 h to demonstrate MMP-9 activity. (B) Zymogram was incubated for 42 h to demonstrate MMP-2 activity. (C) Quantitative analysis of gelatin zymography demonstrates increased MMP-9, but not increased MMP-2 levels in *PAI-1*$^{-/-}$ mice compared to WT animals 3 weeks after BDL. Data represent means ± S.E.M. for 5–7 BDL mice. $^{*}P < 0.01$ versus WT.

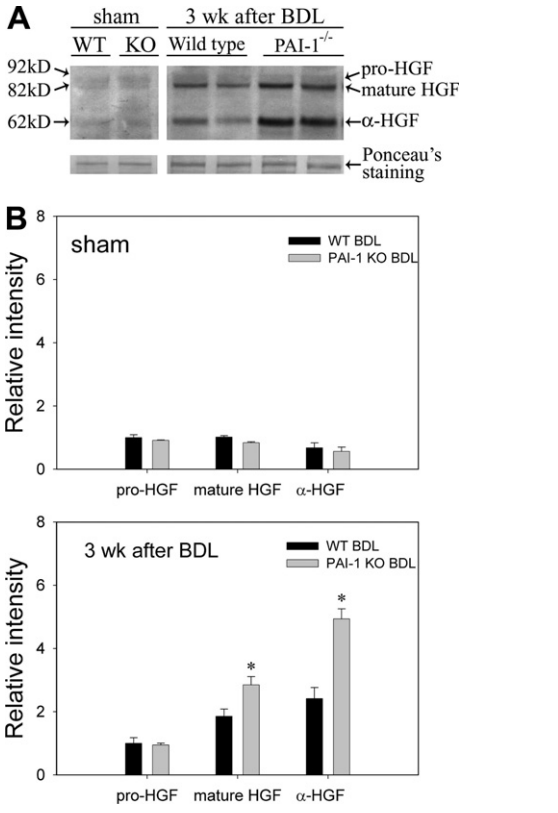

Fig. 6. Protein immunoblot analysis demonstrates increased mature and α-HGF in *PAI-1*$^{-/-}$ mouse liver after BDL compared to WT mice. (A) Liver homogenates were analyzed by immunoblot with an antibody to α-HGF. Bands representing pro-HGF, non-reduced α,β-chain mature HGF, and α-chain HGF (reduced mature HGF) are indicated. (B) Quantitative analysis of HGF at 3 weeks after BDL demonstrated increased mature α-HGF in *PAI-1*$^{-/-}$ mice. HGF levels were equivalent in WT and *PAI-1*$^{-/-}$ mice after sham surgery. Data represent means ± S.E.M. for 3–5 sham and 5–7 BDL mice. $^{*}P \leqslant 0.02$ versus WT at the same time point.

3.6. PAI-1 deficiency increases hepatocyte growth factor (HGF) activation

Our recent analysis of early events after BDL in *PAI-1*$^{-/-}$ mice demonstrated increased HGF activation in mutant animals [2]. To determine if increased HGF activation persisted 3 weeks after BDL in *PAI-1*$^{-/-}$ mice, we performed protein immunoblot analysis (Fig. 6). These studies confirmed elevated active HGF in *PAI-1*$^{-/-}$ compared to WT mice after BDL, but normal levels of active HGF after sham surgery. This increase in active HGF may in part explain the increased pro-MMP9 levels [17,18] observed in *PAI-1*$^{-/-}$ mice.

4. Discussion

Liver fibrosis represents a wound-healing process characterized by stellate cell activation and collagen deposition in response to a variety of chronic injury stimuli [19,20]. Collagen deposition reflects the balance between production and degradation. PAI-1 could potentially influence both collagen production and collagen degradation by controlling proteolytic processing of key regulatory molecules and the ECM. For several reasons we hypothesized that PAI-1 deficiency would increase collagen production. First, PAI-1 deficiency was

anticipated to increase both uPA and tPA activity. This, in turn, should have increased conversion of plasminogen to plasmin [3]. Because plasmin is a prominent TGF-β1 activator and TGF-β1 stimulates stellate cell activation and collagen production [21,22], we predicted increased plasmin activity, increased TGF-β1 activation, increased stellate cell activation, and increased collagen production in *PAI-1*$^{-/-}$ compared to WT mice after BDL. All of these predictions were proved wrong in this study. In contrast, we also predicted increased tPA proteolytic activity would activate HGF and matrix metalloproteinases that could reduce hepatic fibrosis after BDL. These predictions were demonstrated correct in this report.

In agreement with an earlier study indicating that collagen I alpha 1 mRNA levels were equivalent in *PAI-1*$^{-/-}$ and WT shortly after BDL [8], we also found comparable levels of collagen I alpha 1 mRNA in *PAI-1*$^{-/-}$ and WT mice 3 weeks after BDL. Therefore, the simplest explanation for reduced collagen accumulation in *PAI-1*$^{-/-}$ mice, is that collagen degradation is increased by PAI-1 deficiency. While ECM remodeling is a complex process involving a variety of proteases and protease inhibitors [23], we focused on MMP2 and MMP9 because they have altered expression after hepatic injury and have been hypothesized to influence fibrosis [24–27]. Furthermore, their activity is influenced by plasminogen activators. Our studies directly demonstrate increased pro-MMP9 and MMP9 in *PAI-1*$^{-/-}$ compared to WT mice after BDL. In contrast, pro-MMP2 and MMP2 activity were equivalent in *PAI-1*$^{-/-}$ and WT mice. These observations are consistent with known effects of tPA on MMP9 activation [14]. In contrast uPA influences both MMP2 and MMP9 [15,28]. Thus, the lack of MMP2 elevation in our studies is consistent with the similar uPA activity in *PAI-1*$^{-/-}$ and WT mice. One additional explanation for the increase in pro-MMP9 in *PAI-1*$^{-/-}$ mice is the previously described ability of HGF to increase MMP9 synthesis [17] and increased HGF activation in *PAI-1*$^{-/-}$ mice.

Our new data demonstrating that uPA and plasmin activities are equivalent in WT and *PAI-1*$^{-/-}$ 3 weeks after BDL, but tPA activity is elevated are also consistent with our previous observation that uPA and plasmin activities were similar at 72 h [2,8] and 1 week [2] after BDL. In contrast, another group recently reported elevated uPA activity levels in *PAI-1*$^{-/-}$ compared to WT mice 2 weeks after BDL [8]. While the explanation for these discrepant findings is not clear, similar effects of PAI-1 deficiency on tPA, but not uPA were also observed in a renal injury model [7,29] and an arthritis model [30] suggesting that factors other than PAI-1 critically regulate plasmin and uPA at least in some settings. For example, plasmin is also regulated by Serpinf2 [31]. Given the equivalent levels of plasmin activity in *PAI-1*$^{-/-}$ compared to WT mice after BDL, the finding that TGF-β1 activity, stellate cell activation and collagen production were equivalent in *PAI-1*$^{-/-}$ and WT mice is more easily understood because plasmin is one of the more important TGF-β1 activators.

Interestingly, the reduction in fibrosis in *PAI-1*$^{-/-}$ mice after BDL in this report appears less dramatic than the reduction in fibrosis in other model systems [6–8]. Furthermore, blocking PAI-1 is not always protective. For example, *PAI-1*$^{-/-}$ mice have dramatically more severe renal injury than WT animals in a passive anti-glomerular basement membrane glomerulonephritis model [32]. In this case, PAI-1 deficiency increases both uPA and tPA activity. Furthermore, both tPA and uPA can activate latent TGF-β in vitro [32]. Thus, the effect of PAI-1 deficiency on both injury and repair may critically depend on the type of injury and organ affected. This is not surprising since distinct regulatory systems may be more important in some tissues than others and mechanisms controlling fibrosis are complex. For example, latent TGF-β1 is activated not only by tPA, uPA and plasmin, but also by MMP2, MMP9, thrombospondin-1, and by some integrins ($\alpha_v\beta_6$ and $\alpha_v\beta_8$) [33]. Thus, the effect of altering PAI-1 depends on cellular context and the expression of other key regulatory molecules.

Together these studies suggest PAI-1 inhibition may be useful as part of a strategy to prevent cirrhosis resulting from biliary tract obstruction. Because our study has shown that blocking PAI-1 increases HGF activation and reduces fibrosis by increasing ECM degradation, this approach might be particularly useful in combination with strategies to reduce stellate cell activation and collagen synthesis. Furthermore, while several recent approaches to preventing fibrosis have focused on increasing uPA [16,28,34], these new data suggest that increased tPA activity may also prevent fibrosis and that tPA and uPA have distinct roles in controlling liver injury after biliary tract obstruction. Consistent with this result, we also have data to demonstrate a critical role for tPA in reducing hepatic injury after BDL [35]. These analyses not only demonstrated that tPA deficiency increases hepatic injury, but also demonstrated that essentially all of the early protective effects of PAI-1 deficiency after BDL in mice could be prevented by blocking tPA.

Acknowledgements: The authors thank Dr. Jonathan Gitlin, Dr. Phillip Tarr and Dr. David Rudnick (Washington University in St Louis, MO) for helpful comments on the manuscript and Dr. Anthony J. Demetris and Dr. John G. Lunz III (University of Pittsburgh, Pittsburgh, PA) for technical instruction in bile duct ligation of mice. This work was supported by NIH Grants DK62452, DK068371, DK64592, DK57038 to R.O.H. and by the Digestive Disease Research Center Core (DDRCC) Morphology Core (NIH P30-DK52574). The work cited in this publication was performed in a facility supported by NCRR grant C06 RR015502.

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