Carbon tetrachloride-induced liver injury and fibrosis correlates with osteopontin expression in mice

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Abstract

Background and Aims: Osteopontin (OPN) is a multifunctional matrix-anchored cytokine that plays a significant role in innate immunity, cell survival, tumor invasion, and metastasis. We have previously shown that OPN promotes activation of quiescent hepatic stellate cells and increases collagen I expression and deposition. Here, we elucidated the role of OPN in the pathogenesis of hepatic fibrosis in vivo using both OPN transgenic (Opn Tg) and OPN knockout (Opn−/−) mice.

Methods: Liver fibrosis was induced in C57BL/6 WT, Opn Tg and Opn−/− mice by i.p. injections of carbon tetrachloride twice a week for 1 month (5 µl CC14/10 g b.w.t.). This induces significant oxidative stress via generation of CCl3 radical. Commercially available kits were used for biochemical assays. H&E staining and immunohistochemistry were carried out to determine the extent of liver injury. Samples were scored by an experienced hepatopathologist.

Results: To decipher the role of OPN in progressive liver injury, we tested whether liver injury and fibrosis under chronic CCl₄ administration could correlate with OPN expression. WT mice under CCl₄ treatment showed marked elevation of serum AST, ALT and γ-GT, along with striking hepatic inflammation, necrosis, ballooning, activity score, activation of hepatic stellate cells, and scarring. All these pathological changes were significantly elevated by CCl₄ in Opn Tg but were attenuated in Opn−/− mice compared to WT mice. There was up-regulation of collagen I and OPN proteins in CCl₄-treated Opn Tg mice, which was opposite in Opn−/− mice, compared to CCl₄-treated WT mice. Opn Tg mice injected with CCl₄ showed elevated collagenous proteins, portal fibrosis, bridging fibrosis, collagen I band thickness, and fibrosis score than CCl₄-injected WT or Opn−/− mice. Immunohistochemical analysis revealed massive induction of OPN in the liver of control mice. OPN−/− cells were organized in small nests or arborizing duct-like structures, while isolated cells were found at some distance from portal tracts.

Conclusions: These results suggest that OPN plays a significant role in the pathogenesis of hepatic fibrosis in vivo, thus, opening up the possibility of blocking OPN for preventing the development of liver fibrosis.

Introduction

Hepatic fibrosis is characterized by excessive synthesis and deposition of connective tissue components especially fibrillar collagens in the extracellular matrix of the liver. The fibrosis is the result of unbalanced and impaired wound healing responses due to tissue damage such as alcohol, viral hepatitis, nonalcoholic steatohepatitis (NASH) and metabolic disorders. Uncontrolled fibrosis leads to the distortion of normal hepatic architecture and development of nodular and irreversible liver cirrhosis. The pathogenesis of hepatic fibrosis is a dynamic process involving several cell types that include hepatocytes, hepatic progenitor cells (oval cells), Kupffer cells, endothelial cells, hepatic ductal, hepatic myoblasts and biliary epithelial cells. In the fibrogenesis milieu, there is up-regulation of several molecules and proteins, synthesis and release of numerous cytokines and growth factors and a perpetual damage and repair of the liver tissue that lead to fibrosis or cirrhosis. Osteopontin (OPN) is an extracellular matrix protein and was first reported in 1986 in osteoblasts. OPN undergoes extensive posttranslational modifications and its apparent molecular weight would be 44 kDa in above tissue. OPN upregulates in almost all forms of cancer and express splice variants such as OPN-α, β and γ. OPN is expressed in a variety of cells including fibroblasts, macrophages, dendritic cells, endothelial cells, and smooth muscle cells. We have observed that during hepatic fibrogenesis OPN is highly expressed in hepatic stellate cells, biliary epithelial cells and oval cells. The present investigation was aimed to study the role of OPN during the pathogenesis of hepatic fibrosis using wild type, OPN transgenic (Opn Tg) and OPN knockout (Opn−/−) mouse models.

Methods

Acute CCl₄ administration to WT, Opn transgenic and Opn−/− mice

WT, Opn Tg and Opn−/− mice were injected CCl₄ intraperitoneally (5 µl CC14/10 g body weight) and were sacrificed at 48 h. Serum were collected and analysed for ALT and AST to determine the extent of liver injury.

Figure 1. Model of acute drug-induced liver injury. WT, Opn Tg and Opn−/− mice were injected CCl₄ intraperitoneally and were sacrificed at 48 h. Serum were collected and analysed for ALT and AST to determine the extent of liver injury.

Enzyme activities in acute drug-induced liver injury

Serum alanine transaminase (ALT), aspartate transaminase (AST) and γ-glutamyl transpeptidase (γ-GT) activities were measured in the sera. ALT, AST and γ-GT levels were significantly elevated at 48 h in WT mice. Activities were markedly higher in Opn Tg mice and notably decreased in Opn−/− mice compared to WT mice. Mineral oil did not cause any alteration in serum ALT, AST and γ-GT levels. (P < 0.001 compared to the respective untreated control mice in each group and #P < 0.001 compared to CCl₄ treated WT mice, N=6).

Figure 3. Enzyme activities in acute drug-induced liver injury. Serum alanine transaminase (ALT), aspartate transaminase (AST) and γ-glutamyl transpeptidase (γ-GT) activities were measured in the sera. ALT, AST and γ-GT levels were significantly elevated at 48 h in WT mice. Activities were markedly higher in Opn Tg mice and notably decreased in Opn−/− mice compared to WT mice. Mineral oil did not cause any alteration in serum ALT, AST and γ-GT levels. (P < 0.001 compared to the respective untreated control mice in each group and #P < 0.001 compared to CCl₄ treated WT mice, N=6).

Opn−/− mice after acute administration of CCl₄ for 1 month. Administration of CCl₄ resulted in hepatic inflammation, necrosis, ballooning and scarring. All these changes were exacerbated in Opn Tg mice but were attenuated in Opn−/− mice. Mineral oil did not cause any significant changes in any group. The data are representative of 6 mice in each group.

Figure 4. Enzyme activities in chronic drug-induced liver injury. Serum alanine transaminase (ALT), aspartate transaminase (AST) and γ-glutamyl transpeptidase (γ-GT) activities were measured in the sera. ALT, AST and γ-GT levels were significantly higher in CCl₄-treated WT mice but lower in Opn−/− mice compared to WT mice. Mineral oil alone did not increase basal ALT, AST and γ-GT activities. (P < 0.001 compared to respective untreated control mice in each group and #P < 0.001 compared to CCl₄ treated WT mice, N=b).

Conclusions

Chronic administration of CCl₄ resulted in increased injury and liver fibrosis in Opn Tg mice compared to WT mice. Conversely, Opn−/− mice showed less injury and fibrosis than WT mice as depicted below.

Inflammation

Lipid profile

Collagen I

OPN

Our data suggest that OPN plays a significant role in the pathogenesis of hepatic fibrosis and blocking OPN could open the possibility to prevent progression of liver fibrosis.

Figure 5. Opn expression is induced by acute and chronic CCl₄ treatment. WT mice were injected with CCl₄ at a dose of 5 µl/10 g body weight and were sacrificed at 24 and 48 h (acute drug-induced liver injury) or were injected CCl₄ at a dose of 5 µl/10 g body weight for 1 month (chronic drug-induced liver injury).

Figure 6. H&E staining in chronic drug-induced liver injury. H&E staining on liver sections from WT, Opn Tg and Opn−/− mice after chronic administration of CCl₄ for 1 month. Administration of CCl₄ resulted in significant fibrosis in WT (D). The extent of the fibrogenic response was greater in Opn Tg mice with significant perilobular fibrosis (E) compared to WT mice. Conversely, Opn−/− mice showed a reduced fibrogenic response compared to WT mice (F). The data are representative of 6 mice in each group.

Figure 7. Sirius red/Fast green staining in chronic drug-induced liver injury. Sirius red/Fast green staining was performed on liver sections from WT, Opn Tg and Opn−/− mice after chronic CCl₄ administration for 1 month. Liver sections from WT, Opn Tg and Opn−/− mice treated with mineral oil did not show an increase in sinusoidal or perilobular collagen (A, B & C). Chronic administration of CCl₄ resulted in significant fibrosis in WT mice (D). The extent of the fibrogenic response was greater in Opn Tg mice with significant perilobular fibrosis (E) compared to WT mice. Conversely, Opn−/− mice showed a reduced fibrogenic response compared to WT mice (F). The data are representative of 6 mice in each group.

Figure 8. Collagen I immunohistochemistry in chronic drug-induced liver injury. Collagen I immunohistochemistry in liver sections from WT, Opn Tg and Opn−/− mice after chronic CCl₄ administration for 1 month. Liver sections from WT, Opn Tg and Opn−/− mice treated with mineral oil did not show an increase in sinusoidal or perilobular collagen (A, B & C). Chronic administration of CCl₄ resulted in significant fibrosis in WT mice (D). The extent of the fibrogenic response was greater in Opn Tg mice with significant perilobular fibrosis (E) compared to WT mice. Conversely, Opn−/− mice showed a reduced fibrogenic response compared to WT mice (F). The data are representative of 6 mice in each group.

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