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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 matricellular 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 secretion. Here, we elucidated the role of OPN in the pathogenesis of hepatic fibrosis *in vivo* using both OPN transgenic mice (*Opn* Tg) and OPN knockout mice *(Opn–/–)*.

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 CCl4/10 g b. wt.), which 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 γGT, along with striking hepatic inflammation, necrosis, ballooning, activity score, activation of hepatic stellate cells, and scarring. All these pathophysiological markers were significantly elevated by CCl4 in *Opn* Tg mice 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, while the opposite occurred in *Opn–/–* mice, compared to CCl4-treated WT mice. *Opn* Tg mice injected with CCl4 showed elevated collagenous proteins, portal fibrosis, bridging fibrosis, greater collagen I band thickness, and fibrosis score than CCl4-injected WT or *Opn–/–* mice. Immunohistochemical analysis revealed massive induction of OPN in biliary epithelial cells, oval cells, and hepatic stellate cells under CCl4 treatment in WT and *Opn* Tg 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. Fibrosis is the result of unbalanced and impaired wound healing response due to chronic stimuli such as alcoholism, 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), stellate cells, Kupffer cells, endothelial cells, hepatic dendritic cells, hepatic myofibroblasts and biliary epithelial cells. In the fibrogenesis *milieu,* there is upregulation 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 and cirrhosis.

Osteopontin (OPN) is an extracellular matrix protein and was first reported in 1986 in osteoblasts. OPN undergo extensive posttranslational modifications and its apparent molecular weight would be 44 kDa or above depends on the tissue or origin. OPN upregulates in almost all forms of cancer and express splice variants such as OPN-a, b and c. OPN is expressed in a variety of cells including fibroblasts, macrophages, dendritic ells, 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

Figure 1

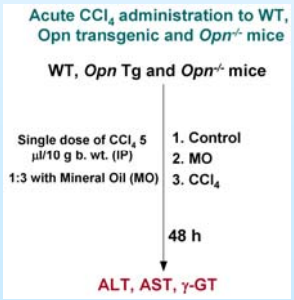

Figure 1. Model of acute drug-induced liver injury. WT, *Opn* Tg and *Opn-/-* mice were injected CCl4 intraperitoneally (5 µl CCl_4/10 g of body weight) and were sacrificed at 48 h. Sera were collected and analyzed for ALT, AST and γ-GT activities to determine the extent of liver injury.

Figure 2

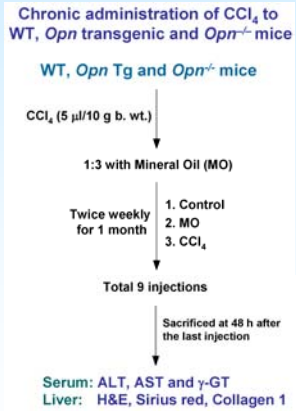

Figure 2. Model of chronic drug-induced liver injury. WT, *Opn* Tg and *Opn-/-* mice were injected CCl_4 intraperitoneally twice a week (5 µl CCl_4/10 g body weight) and were sacrificed at 48 h after the last dose. Sera were collected and analyzed for ALT, AST and γ-GT activities to determine the extent of liver injury. Livers were removed for histopathology and immunohistochemistry.

Results

Figure 3

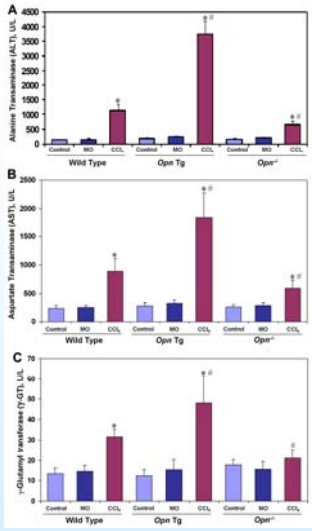

Figure 3. Enzyme activities in acute drug-induced liver injury. Serum alanine transaminase (ALT), aspartate transaminase (AST) and γ-glutamyl transpeptidase (γ-GT) activities in WT, *Opn* Tg and *Opn-/-* mice after a single dose of CCl_4 (5 µl/10 g of body wt.). Serum ALT, AST and γ-GT levels were significantly 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 CCl_4 treated WT mice, n=6).

Figure 4

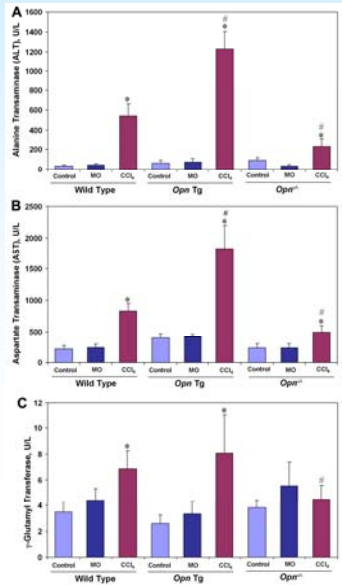

Figure 4. Enzyme activities in chronic drug-induced liver injury. Serum alanine transaminase (ALT), aspartate transaminase (AST) and γ-glutamyl transpeptidase (γ-GT) activities in WT, *Opn* Tg and *Opn-/-* mice after CCl_4 for 1 month (5 µl/10 g of body weight). Serum ALT, AST and γ-GT levels were significantly elevated after chronic CCl_4 treatment in WT mice. Enzyme activities were significantly higher in CCl_4-treated *Opn* Tg 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 CCl_4 treated WT mice, N=6).

Figure 5

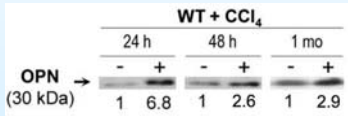

Figure 5. OPN expression is induced by acute and chronic CCl_4 treatment. WT mice were injected with CCl_4 at a dose of 5 µl/10 g body weight and were sacrificed at 24 h and 48 h (acute drug-induced liver injury) or were injected CCl_4 at a dose of 5 µl/10 g body weight for 1 month (chronic drug-induced liver injury).

Figure 6

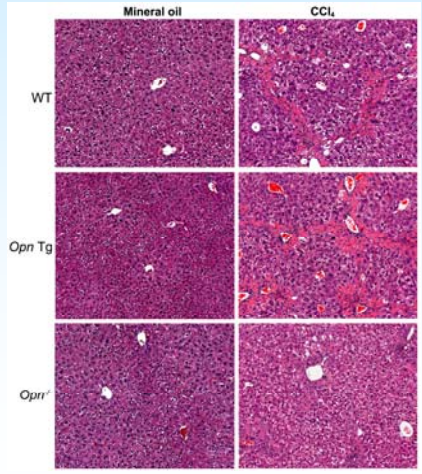

Figure 6. H&E staining in chronic drug-induced liver injury. H&E staining on liver sections of WT, *Opn* Tg and *Opn-/-* mice after chronic administration of CCl_4 for 1 month. Administration of CCl_4 resulted in hepatic inflammation, necrosis, ballooning and scarring. All these changes were exacerbated 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 7

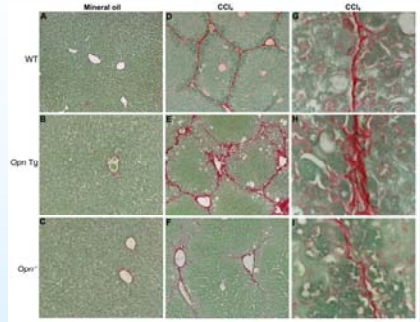

Figure 7. Sirius red/Fast green staining in chronic drug-induced liver injury. Sirius red/Fast green staining for total collagens on liver sections from WT, *Opn* Tg and *Opn-/-* mice after chronic administration of CCl_4 for 1 month. Mineral oil did not induce collagen staining in WT, *Opn* Tg and *Opn-/-* mice (A, B & C). CCl_4 induced fibrosis in WT mice (D), which was exacerbated in *Opn* Tg mice (E) and notably attenuated in *Opn-/-* mice (E). At higher magnification (x650), the Sirius red/Fast green staining depicted thicker collagen fibers in *Opn* Tg mice (H) compared to WT mice (G), and the collagen fibers were thinner in *Opn-/-* mice than in WT mice (I). The data are representative of 6 mice in each group.

Figure 8

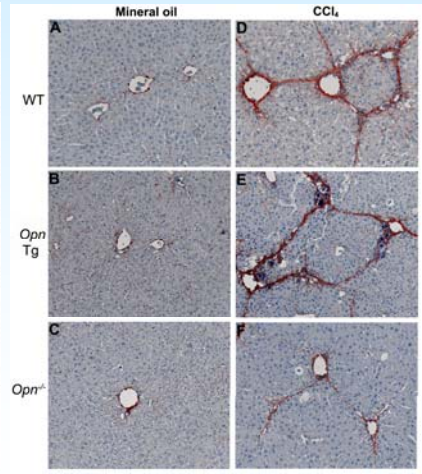

Figure 8. Collagen I immunohistochemistry in chronic drug-induced liver injury. Collagen I immunohistochemistry in liver sections from WT, *Opn* Tg and *Opn-/-* mice after chronic CCl_4 administration for 1 month. Liver sections from WT, *Opn* Tg and *Opn-/-* mice treated with mineral oil did not show an increase in sinusoidal or periportal collagen I (A, B & C). Chronic administration of CCl_4 resulted in significant fibrosis in WT mice (D). The extent of the fibrogenic response was greater in *Opn* Tg mice with significant periportal fibrosis (E) compared to WT mice. Conversely, *Opn-/-* mice showed a reduced pro-fibrogenic response compared to WT mice (F). The data are representative of 6 mice in each group.

Conclusions

Chronic administration of CCl_4 resulted in increased injury and liver fibrosis in Opn Tg mice compared WT mice. Conversely, $Opn^{-/-}$ mice shown less injury and fibrosis than WT mice as depicted below.

↓ Inflammation

↓ AST/ALT/ γ-GT

↓ Liver pathology (H&E)

↓ 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.

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