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Interfering of connective tissue growth factor mRNA protects N-nitrosodimethylamine induced toxic liver injury in rats

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Abstract

Connective tissue growth factor (CTGF) is a profibrogenic molecule and plays a crucial role in the pathogenesis of hepatic fibrosis. CTGF is dramatically upregulated in toxic liver injury including alcoholic fibrosis. The aim of our present investigation was to examine whether interference of CTGF at the mRNA level could prevent the progression of NDMA-induced hepatic fibrosis in rats. Liver injury was induced by intraperitoneal injections of *N*-nitrosodimethylamine (NDMA) in adult male albino rats in doses of 10 mg/kg body weight daily for seven consecutive days. The animals were left for an additional 7 days without any treatment. Another set of animals received intraperitoneal injections of a mammalian expression vector carrying CTGF siRNA cDNA in doses of 1 mg DNA/kg body weight, daily 2 h prior to the administration of NDMA and afterwards every day until the sacrifice of the animals on day 14. Serial administrations of NDMA resulted in activation of hepatic stellate cells, upregulation of CTGF and TGF-β1 both at mRNA and protein levels and well developed fibrosis in the liver. CTGF siRNA treated animals showed marked decrease of hepatic stellate cell activation, downregulation of CTGF and TGF-β1 both at mRNA and protein levels, remarkable reduction in fibrosis and deposition of collagen fibers in the liver and significant decrease of serum hyaluronic acid and TGF-β1. Our study demonstrated that knockdown of CTGF mRNA has potential therapeutic application to prevent hepatic fibrogenesis. ***(Gene Therapy 2007; 14: 790-803).***

Introduction

Hepatic fibrosis is a dynamic process that involves the interplay of different cell types in the hepatic tissue. The transformation of quiescent hepatic stellate cells into myofibroblast-like cells with the expression of smooth muscle actin filaments initiates the chronic process of hepatic fibrosis that may end with the fatal stage of liver cirrhosis. A cascade of signaling and transcriptional events in the activated stellate cells underlies the pathogenesis of hepatic fibrosis.

Connective tissue growth factor (CTGF) is a multifunctional protein involved in the regulation of cell growth and tissue remodeling. CTGF plays a key role in the pathogenesis of hepatic fibrosis and stimulates the transformation of resting hepatic stellate cells into myofibroblasts, which leads to the production of more CTGF. CTGF also stimulates the production of collagens, fibronectin and laminin, the predominant molecules of the extracellular matrix (ECM) of the liver. The inhibition of CTGF-mediated hepatic stellate cell activation and the related ECM production may be a promising strategy to prevent hepatic fibrosis and alcoholic cirrhosis.

Materials and Methods

The toxic liver injury was induced by serial intraperitoneal injections of N-nitrosodimethylamine (NDMA) in doses of 10 mg/kg body weight daily for seven consecutive days. The siRNA group of animals received intraperitoneal injections of CTGF siRNA plasmid vector in doses of 1 mg DNA/kg body mass daily 2 h prior to the administration of NDMA and afterwards daily until the sacrifice of the animals on day 14. Another group of animals received scrambled CTGF siRNA plasmid vector daily for up to 14 days.

The pathogenesis of NDMA-induced hepatic fibrosis and the effects of CTGF siRNA were evaluated through hematoxylin and eosin as well as Masson's trichrome staining. The activation of hepatic stellate cells demonstrated through immunohistochemical staining of α-smooth muscle actin (α-SMA) filaments is also considered as a marker for the degree of hepatic fibrosis and also as the effects of CTGF siRNA for the inhibition of fibrogenesis. CTGF and TGF-β1 mRNA and protein levels were determined in the hepatic tissue. Serum hyaluronic acid (HA) and TGF-β1 levels were also measured.

Figure 1

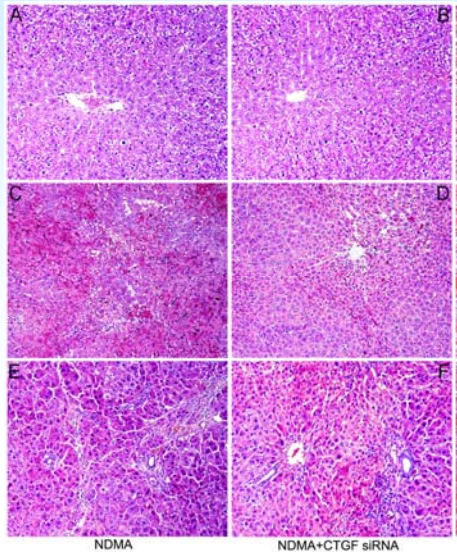

Figure 1. H&E staining of rat liver during NDMA-induced hepatic fibrosis and after concurrent treatment with CTGF siRNA. (a) Normal liver (×100). (b) CTGF siRNA control liver (×100). Scrambled vector was administered for 14 days. (c) NDMA was administered daily in doses of 10 mg/kg body weight for 7 consecutive days (×100). Massive hepatic necrosis. (d) CTGF iRNA was administered daily 2 h before the administration of NDMA (×100). Focal necrosis. (e) Day 14 after the start of daily administration of NDMA for 7 consecutive days (×100). Marked hepatic fibrosis. (f) Day 14 (×100). CTGF siRNA was administered daily until day 14 after the start of the administration of NDMA for 7 consecutive days. Moderate necrosis.

Figure 2

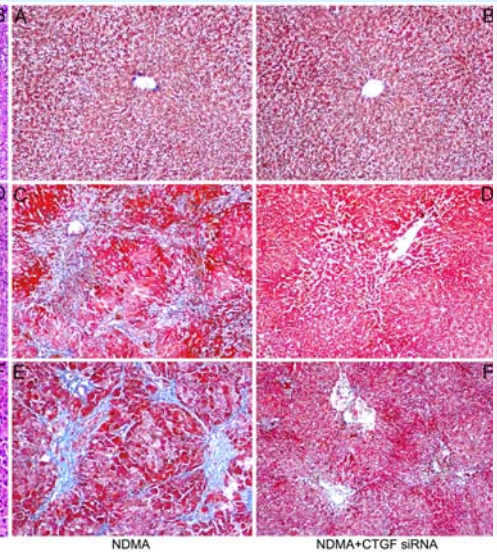

Figure 2. Masson's trichrome staining of rat liver during NDMA-induced hepatic fibrosis and after concurrent treatment with CTGF siRNA. (a) Normal liver (×100). (b) CTGF siRNA control liver (×100). Scrambled vector was administered for 14 days. (c) NDMA was administered daily in doses of 10 mg/kg body weight for 7 consecutive days (×100). Massive hepatic necrosis with early fibrosis. (d) CTGF siRNA was administered daily 2 h prior to the administration of NDMA (×100). Moderate necrosis. (e) Day 14 after the start of daily administration of NDMA for 7 consecutive days (×100). Marked hepatic fibrosis. (f) Day 14 (×100). CTGF siRNA was administered daily until day 14 after the start of daily administration of NDMA for 7 consecutive days. Moderate marked necrosis.

Figure 3

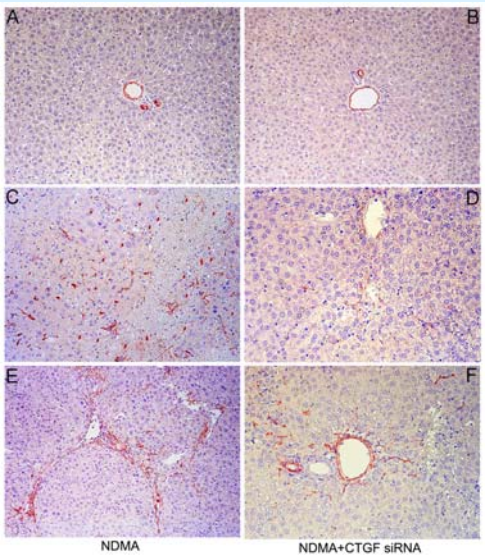

Figure 3. Immunohistochemical staining for α-SMA. (a) Normal liver (×200). The absence of α-SMA staining. (b) CTGF scrambled siRNA (×200). The absence of α-SMA staining. (c) NDMA was administered daily in doses of 10 mg/kg body weight for 7 consecutive days (×200). Abundant staining of α-SMA demonstrating large number of activated stellate cells especially in the necrotic zone. (d) CTGF siRNA was administered daily 2 h prior to the administration of NDMA (×200). A few activated stellate cells in the necrotic areas. (e) Day 14 after the start of daily administration of NDMA for 7 consecutive days (×100). Remarkable staining of α-SMA demonstrating enormous number of activated stellate cells especially in the fibrotic areas. (f) Day 14 (×200). CTGF siRNA was administered daily until day 14 after the start of NDMA administration. Staining of few activated stellate cells in the necrotic areas.

Figure 4

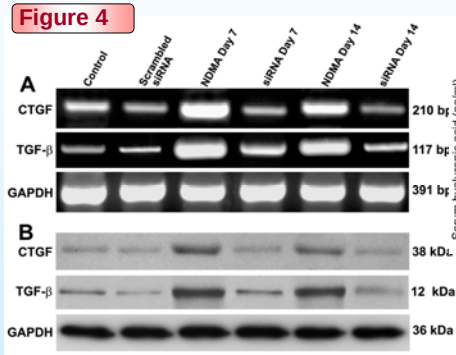

Figure 4. (**A**) Semiquantitative RT–PCR for the expression of CTGF and TGF-β mRNA. The total cellular RNA was isolated using Qiagen RNeasy kit. Glyceraldehyde-3-phosphate dehydrogenase mRNA expression was used as a housekeeping gene. (**B**) Western blot analysis for CTGF and TGF-β in the rat liver during NDMA-induced hepatic fibrosis and also after simultaneous treatment with CTGF siRNA. The nitrocellulose membranes were re-probed and analyzed for GAPDH content to demonstrate that similar amounts of protein had been loaded in each lane.

Figure 5

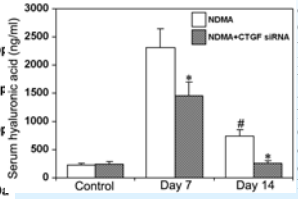

Figure 5. Serum HA during NDMA-induced hepatic fibrosis and also after concurrent treatment with CTGF siRNA. Serum HA was determined using an ELISA-based sandwich HA-binding protein assay kit (*P < 0.001 when compared with NDMA samples without CTGF siRNA on respective days and $^{\#}P$ < 0.001 when compared with respective NDMA values on day 7)

Figure 6

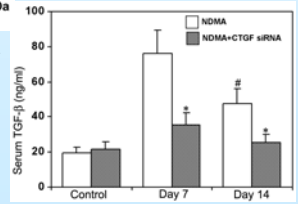

Figure 6. TGF-β levels in the rat serum during NDMA-induced hepatic fibrosis and also after concurrent treatment with CTGF siRNA. The TGF-β in rat serum was determined using an enzyme immunoassay kit (*P < 0.001 when compared with NDMA samples without CTGF siRNA on respective days and $^{\#}P$ < 0.01 when compared with respective NDMA values on day 7).

Conclusions

- **Serial administrations of NDMA produced well developed fibrosis in rat liver within 14 days.**
- **NDMA treatment resulted in activation of hepatic stellate cells, upregulation of CTGF and TGF-β1 both at mRNA and protein levels.**
- **Treatment with CTGF siRNA during NDMA administrations showed marked decrease in hepatic stellate cell activation, downregulation of CTGF and TGF-β both at mRNA and protein levels.**
- **Downregulation of CTGF resulted in remarkable reduction in fibrosis and deposition of collagen fibers in the liver as well as decrease of serum HA and TGF-β.**
- **Knockdown of CTGF mRNA has potential therapeutic application to prevent hepatic fibrogenesis.**

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