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 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. Administration 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.

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 collagen, 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 μg 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. Serum hyaluronic acid (HA) and TGF-β1 levels were also measured.

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.