

AASLD 2009 #1111 MMP-13 Deletion Protects Monocrotaline Induced Liver Injury in Mice

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Abstract

Monocrotaline (MCT) is both hepatotoxic and pneumotoxic in several species including human. Since matrix metalloproteinases-1 (MMP-1) is absent in mice, MMP-13 is responsible for cleavage and maturation of connective tissue growth factor (CTGF). This study was aimed to elucidate the molecular events associated with hepatic fibrogenesis in MCT induced toxic liver injury. Hepatic injury was produced through subcutaneous administrations of MCT in doses of 60 mg/100 g body weight once a week for 4 weeks. Serial administrations of MCT resulted in marked elevation of AST, ALT, and hyaluronic acid (HA) in the serum and extensive activation of hepatic stellate cells, massive hepatic necrosis, bridging fibrosis and deposition of collagen fibers in the liver. However, these changes were significantly attenuated in MCT administered MMP-13 knockout mice. Staining for CTGF showed marked upregulation in MCT-treated wild-type mice but not in the knockout. Semiquantitative and real-time RT-PCR for CTGF, TGF- β 1, and type-I collagen mRNA showed remarkable upregulation in MCT administered wild-type mice, but not in similarly treated MMP-13 knockout mice. Protein levels of CTGF, TGF- β 1, MMP-2, MMP-9 and type I collagen were also increased in MCT treated wild-type mice. All these molecules were significantly reduced and CTGF was absent in MCT treated MMP-13 knockout mice compared to the similarly treated wild-type. Taken together, these results show that MMP-13 plays a crucial role in the pathogenesis of MCT induced liver injury through activation of CTGF. Furthermore, our study indicates that blocking of CTGF could pave the way for the therapeutic intervention of 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 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- β 1 mRNA and protein levels were determined in the hepatic tissue. Serum hyaluronic acid (HA) and TGF- β 1 levels were also measured.

Figure 1

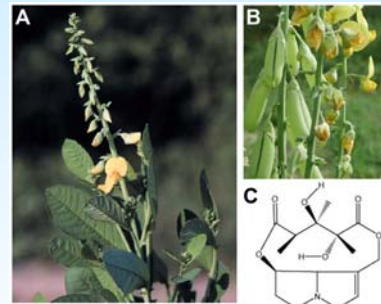


Figure 1. (A) Plant *Crotalaria spectabilis*. (B) Seeds of *Crotalaria spectabilis* rich in monocrotaline. (C) Chemical structure of monocrotaline.

Figure 2

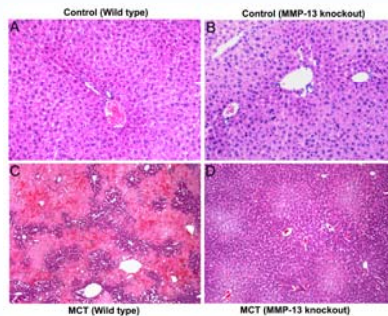


Figure 2. H&E staining of liver tissue in wild type and MMP-13 knockout mice before and after treatment with MCT. The data are representative of 8 mice per group. (A) Wild-type mice (x100). (B) MMP-13 knockout mice (x100). There is no visible pathological alteration in the liver tissue. (C) Wild-type mice (x40). MCT was administered at a dose of 60 mg/100 g body weight once a week for 4 weeks. Massive hepatic necrosis and loss of normal architecture. Severe congestion of hepatic arteries and extensive hemorrhage. (D) MMP-13 knockout mice (x40). MCT was administered at a dose of 60 mg/100 g body weight once a week for 4 weeks. Slight centrilobular necrosis.

Figure 3

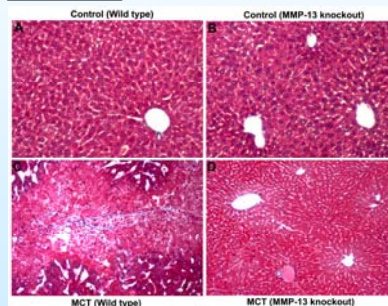


Figure 3. Masson's trichrome staining of liver sections in wild type and MMP-13 knockout mice before and after administration of MCT. The data presented are representative of 8 mice per group. (A) Wild-type mice (x100). (B) MMP-13 knockout mice (x100). Collagen staining is absent. (C) Wild-type mice (x100). MCT was administered at a dose of 60 mg/100 g body weight once a week for 4 weeks. Marked hepatic fibrosis with deposition of fine collagen fibers stained in blue. (D) MMP-13 knockout mice (x100). MCT was administered at a dose of 60 mg/100 g body weight once a week for 4 weeks. Absence of collagen deposition. Initiation of centrilobular necrosis.

Figure 4

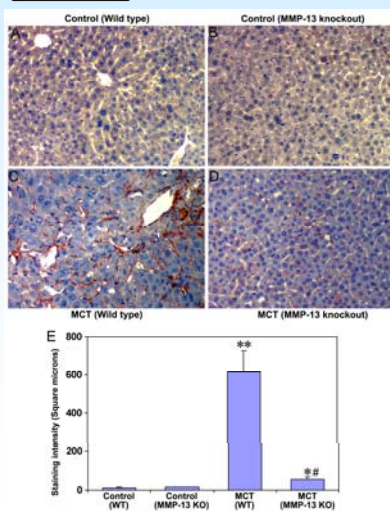


Figure 4. Immunohistochemical staining for α -smooth muscle actin demonstrates activated hepatic stellate cells in wild-type and MMP-13 knockout mice before and after treatment with MCT. The data are representative of 8 mice per group. (A) Wild-type mice (x100). Absence of α -SMA staining. (B) MMP-13 knockout mice (x100). Activation of hepatic stellate cells is not detectable. (C) MCT treated wild-type mice (x100). Extensive staining of α -SMA demonstrating large number of activated stellate cells especially in centrilobular zone. (D) MMP-13 knockout mice (x100). Staining of α -SMA is absent. (E) Quantitative evaluation of activated stellate cells using Image-Pro Plus software. The data are mean \pm S.D. of 8 samples from ten randomly selected microscopic fields (** $P < 0.001$ and * $P < 0.01$ when compared with the respective untreated controls and # $P < 0.001$ compared with the MCT treated wild type).

Figure 5

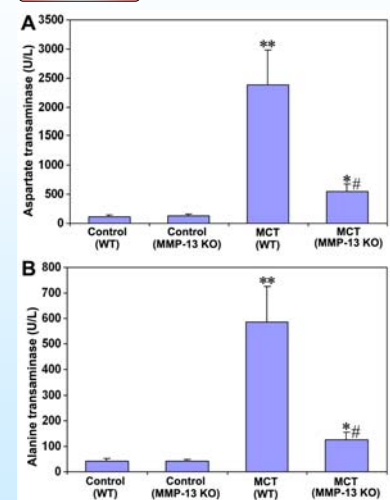


Figure 5. (A) Aspartate transaminase and (B) Alanine transaminase levels in serum in wild type and MMP-13 knockout mice before and after treatment with MCT. The data are mean \pm S.D. of 8 samples (** $P < 0.001$ and * $P < 0.01$ when compared with the respective untreated controls and # $P < 0.001$ compared with the MCT treated wild type).

Figure 6

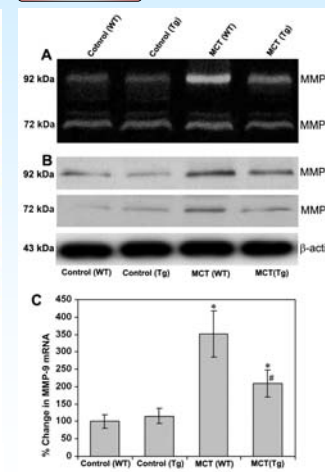


Figure 6. (A) Gelatin-zymography for MMP-9 and MMP-2 in wild-type and MMP-13 knockout mice before and after treatment with MCT. (B) Western blotting for protein levels of MMP-9 and MMP-2 in the liver tissue. β -actin was used as a loading control. (C) Real-time RT-PCR analysis using SYBR green for the quantitative expression of MMP-9 mRNA in wild-type and MMP-13 knockout mice before and after treatment with MCT. The data are mean \pm S.D. of 8 samples (** $P < 0.001$ compared with the respective untreated controls and # $P < 0.001$ compared with the MCT treated wild type).

Figure 7

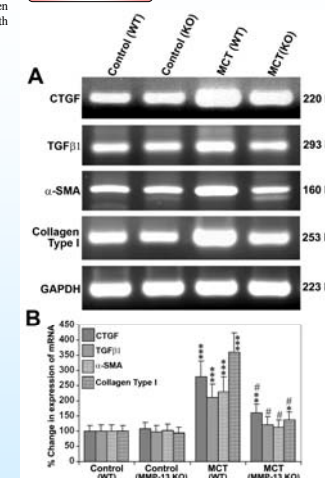


Figure 7. (A) Semiquantitative RT-PCR for the expression of CTGF, TGF- β 1, α -SMA and collagen type I (α 1) mRNA in wild-type and MMP-13 knockout mice before and after treatment with MCT. The total cellular RNA was isolated using Qiagen RNeasy kit. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene. The data are representative of 8 samples. (B) Real-time RT-PCR analysis using SYBR green for the quantitative evaluation of the expression of CTGF, TGF- β 1, α -SMA and collagen type I (α 1) mRNA in wild-type and MMP-13 knockout mice before and after treatment with MCT. The data are mean \pm S.D. of 8 samples (** $P < 0.001$ compared with the respective untreated controls and # $P < 0.001$ compared with the MCT treated wild type).

Figure 8

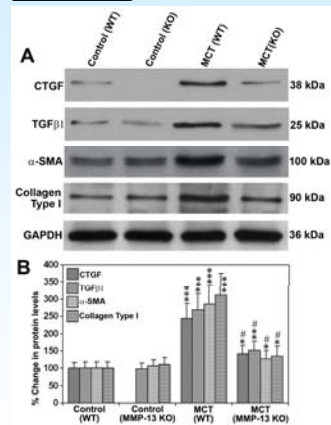


Figure 8. (A) Western blotting for the protein levels of CTGF, TGF- β 1, α -SMA and collagen type I in the liver tissue of wild-type and MMP-13 knockout mice before and after treatment with MCT. The PVDF membranes were reprobed for GAPDH content to demonstrate equal loading. The data are representative of 8 samples. (B) Quantitative evaluation of Western blotting. The Western blot images were quantified using Gel-Pro analyzer software (Media Cybernetics, Silver Spring, MD). The data are mean \pm S.D. of 8 samples (** $P < 0.001$ compared with the respective untreated controls and # $P < 0.001$ compared with the MCT treated wild type).

Conclusions

* Serial administrations of monocrotaline produced centrilobular necrosis and well developed fibrosis in mouse liver within 28 days.

* Treatment with monocrotaline resulted in increased levels of serum AST, ALT and HA, activation of hepatic stellate cells, upregulation of MMP-2, MMP-9, CTGF, TGF- β 1, α -SMA and collagen type I in wild type mice.

* Administration of monocrotaline in MMP-13 knockout mice showed inhibition of fibrosis, marked reduction in the activation of hepatic stellate cells, down regulation of CTGF, TGF- β 1, α -SMA and collagen type I compared to similarly treated wild type mice.

* MMP-13 plays a crucial role in the pathogenesis of hepatic fibrosis through activation of CTGF.

* Effective blocking of CTGF has potential therapeutic implication to prevent hepatic fibrosis.