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Effects of Magnesium Isoglycyrrhizinate on TGF-β/Smad Signal Expression in Hepatic Stellate Cells in Rats

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Objective: To investigation the effects of Magnesium isoglycyrrhizinate (MI) on TGF-β/Smad signal expression in cultured HSC stimulated by TGF-β_1 and then to reveal the possible molecular mechanism of MIG on anti-fibrosis.
Methods: The rat HSC-T6 were cultured with or without MIG (0～10mg/ml) in vitro after TGF-β_1 stimulation. The inhibitory rate of proliferation was measured by MTT method, the viability of cells was tested by evaluation of supernant LDH. The mRNA level of TGF-β_1, Smad3, Smad 7 were assessed by semi-quantitative RT-PCR method.
Results: MTT method showed an obvious inhibition of HSC proliferation after MIG (0–10 mg/ml) treatment. Different concentrations of MIG did not affect the vitality on HSC by LDH measurement. Different concentrations of MIG increased the mRNA level of TGF-β_1, Smad 3, Smad7 in HSC-T6, and showed a certain trend of dose-effect relationship, and the mRNA of TGF-β1 and Smad3, Smad7 expression were consistent changes.
Conclusions: MIG has significant impact on rat hepatic fibrosis liver TGF-β/Smad signaling expression Inhibited rat hepatic stellate cell growth and proliferation, and its mechanism may be reduce the expression of TGF-β1, Smad3, Smad7 mRNA.

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Long-term Transforming Growth Factor-β1 Exposure Induced a Sustained Epithelial-mesenchymal Transition Process in Hepatic Oval Cells with an Increased α-fetoprotein Expression

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Background: Transforming growth factor-β1 (TGF-β1) is the key regulator in the initiation and progression of liver fibrosis, and we have learned TGF-β1 could induce an increased expression of extracellular matrix (ECM) in hepatic progenitors within 2 days through epithelial-mesenchymal transition (EMT). The aim of the present study is to analyze the long-term effects of TGF-β1 exposure on hepatic progenitors in vitro.
Methods: Hepatic oval cells were isolated from rats fed a choline-deficient diet supplemented with ethionine and characterized by flow cytometry. For TGF-β1 treatment, the cells were cultured in 10% FBS-DMEM/F12 medium containing 1ng/ml TGF-β1 for 16 days with the medium replaced every 2 days.
Results: Hepatic oval cells were positive for the progenitor-specific markers OV-6, α-fetoprotein (AFP) and Dlk, as well as hepatocyte marker albumin and cholangiocyte marker cytokeratin 19. In the first four days of TGF-β1 exposure, the cell number gradually reduced, the cell size continuously became larger and more and more cell filaments appeared in the cytoplasm, while the cell number and cell morphology sustained in the following days. Real-time PCR results showed TGF-β1 upregulated snail expression and kept downregulation of e-cadherin, with 3 times more snail and 20% e-cadherin at 16th day, indicating the sustained EMT effects of TGF-β1. Furthermore, TGF-β1 increased the ECM expression by collagen I, collagen III, connective tissue growth factor, and tissue inhibitor of metalloproteinase, in a time-dependent manner, which confirmed the sustained EMT process. Interestingly, the expression of AFP and cytokeratin 19 increased time-dependently to 10 folds and nearly 30 folds, respectively, at 16th day after TGF-β1 exposure.
Conclusion: Long-term TGF-β1 exposure induces a sustained EMT process in hepatic oval cells with increased AFP expression, which might partially interpret the increased serum AFP level in cirrhosis patients.

PP15-37

Erythropoietin Decreases Carbon Tetrachloride-induced Hepatic Fibrosis by Inhibiting Transforming Growth Factor-beta

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Background and aim: In addition to hematopoietic effect, the erythropoietin is known as a multifunctional cytokine with anti-fibrosis and organ-protective activities. The purpose of this study is to evaluate the effect of recombinant human erythropoietin (rhEPO) on hepatic fibrosis and hepatic stellate cells (HSCs).
Methods: Carbon Tetrachloride (CCl_4) induced hepatic fibrosis mice models were used for in vivo study and hepatic stellate cells (HSCs) line for in vitro study. CCl_4 and rhEPO (0, 200 or 1,000 U/kg) was injected intraperitoneally in BALB/c mice three times a week for 4 weeks. Immunohistochemistry and immunoblotting were performed to evaluate expressions of transforming growth factor-β1 (TGF-β1), α-smooth muscle actin (α-SMA), and fibronectin in explanted liver. Immunoblotting of α-SMA, phophorylated Smad-2 and Smad-2/3 was performed in HSCs treated with TGF-β1 and/or rhEPO.
Results: Expressions of TGF-β1, α-SMA, and fibronectin were increased in CCl_4 injected mice livers, but significantly attenuated by co-treatment with CCl_4 and rhEPO. Co-treatment of rhEPO markedly suppressed fibrosis in Masson's trichrome compared to treatment of only CCl_4. By identifying the expression of rhEPO receptors in HSCs by RT-PCR, we confirmed the involvement of rhEPO receptor dependent anti-fibrosis effect in experiment animal models. TGF-β1 increased phosphorylated α-SMA, Smad-2 expressions in HSCs, which were decreased by rhEPO co-treatment.
Conclusions: Treatment of rhEPO effectively suppressed fibrosis in CCl_4-induced liver fibrosis mice models. Anti-fibrosis effect of rhEPO could be related to inhibition of TGF-β1 induced activation of HSCs.

PP15-38

Human MMP-1 Transgene Protects Experimentally Induced Hepatic Fibrosis in Mice

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Hepatic fibrosis is characterized by excessive synthesis and deposition of fibrillar collagens in the liver with impaired turnover. Since the major collagenolytic enzyme, matrix metalloproteinase-1 (MMP-1) is absent in mice, MMP-13 alone is responsible for collagen remodeling during fibrogenesis. In the present study, we examined whether transgenic expression of human MMP-1 could protect the N-nitrosodimethylamine (NDMA) induced hepatic fibrosis in mice. Hepatic fibrosis was induced in wild-type and MMP-1 transgenic mice through intraperitoneal injections of NDMA in doses of 1 mg/100 g body weight on 3 consecutive days of every week over a period of 4 weeks. NDMA administrations resulted in marked elevation of serum AST, ALT, hyaluronic acid (HA), transforming growth factor-b1 (TGF-b1) and procollagen-III peptide in wild-type mice. There was marked activation of hepatic stellate cells, deposition of collagen-1 and HA in the liver. However, these processes were markedly decreased in NDMA administered MMP-1 transgenic mice. qRT-PCR and Western blotting for collagen I, a-smooth muscle actin (a-SMA), and TGF-b1 demonstrated marked upregulation of mRNA and protein levels respectively, in NDMA treated wild-type mice but not in

similarly treated transgenic mice. Our study demonstrated that transgenic expression of MMP-1 protects the liver from NDMA induced hepatic fibrosis in mice by preventing excessive deposition of collagens in the liver. Our results further indicate that methods to upregulate the activity of MMP-1 could provide an option for therapeutic intervention of human hepatic fibrosis.

PP15-39

Nitrix Oxide Plays a Role in Mediating the Effects of Adiponectin on Hepatic Stellate Cell Proliferation, Migration and Apoptosis

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Introduction: Adiponectim ameliorates liver fibrosis in rodent models, but the mechanisms have not been completely elucidated. Nitric oxide (NO) has anti-fibrogenic properties and adiponectin is known to stimulate endothelial Nitric Oxide Synthase (eNOS) and NO production in non-liver cells. We hypothesized that adiponectin may promote NOS expression and NO production in hepatic non-parechymal cells and thereby modulate the anti-fibrogenic effects of adiponectin.
Methods: Rat HSCs, KCs and SECs were isolated and cultured. Recombinant adiponectin (5 µg/mL) was added to HSCs, KCs and SECs for 24 h. For inhibition experiments, adiponectin (5 µg/mL) or adiponectin and the NO inhibitor (L-NAME 1mM) were added to HSCs for 24 h. iNOS gene and protein expression in HSCs and KCs as well as eNOS phosphoprotein expression in SECs were analyzed by qPCR, immunblot and flow cytometry. NO metabolite concentrations (NO_2/NO_3) in culture medium were measured using the NO Assay Kit. HSC proliferation was determined by BrdU incorporation. HSC migration was examined by wound healing assay. HSC apoptosis was assessed by Annexin V flowcytometry and DAPI staining.
Results: Adiponectin enhanced HSC and KC iNOS gene (1,000 and 8,000 fold respectively, $p < 0.0001$) and protein expression (7.2 and 7.7 fold respectively, $p < 0.001$). Adiponectin increased SEC eNOS protein (1.7 fold, $p < 0.05$). As expected, adiponectin increased NO (NO_2/NO_3) concentration in HSC, KC and SEC medium. Adiponectin attenuated HSC proliferation and migration ($p < 0.01$) as well as augmented HSC apoptosis. These effects were partially reversed after L-NAME treatment ($p < 0.01$, 0.05 and 0.01).
Conclusions: Adiponectin up-regulates iNOS/eNOS/NO expression in hepatic nonparechymal cells. Adiponectin inhibits HSC proliferation and migration as well as enhances HSC apoptosis. The latter effects are at least, in part, mediated through adiponectin-induced NO production. Further experiments to determine the signaling pathways which modulate adiponectin-mediated iNOS/eNOS/NO expression are under investigation.

PP15-40

CYP2E1 Inhibitor (DDC) Significantly Upregulates MMP-1 Expression through ERK1/2, p38MAPK and AKT Signaling Pathways

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Background/aims: Cytochrome P450 2E1 (CYP2E1)-derived reactive oxygen species from hepatocyte mediate paracrine stimulation of collagen I protein synthesis by hepatic stellate cells, and CYP2E1 inhibitors block this effect, but the mechanism has not been fully clarified.
Methods: A model was developed using co-cultures of C3A cells, which do express CYP2E1 (C3A-2E1) or do not express CYP2E1 (C3A) with human hepatic stellate cells (LX-2). The co-culture systems were treated by diethyldithiocarbamate (DDC, CYP2E1 inhibitor), and the effects of DDC on collagen I, MMP-1 and activation of MAPK pathway in LX-2 were evaluated.
Results: Compared with LX-2 co-cultured with C3A, both the intracellular H_2O_2 level and collagen I expression increased in LX-2 co-cultured with C3A-2E1, and DDC inhibited the upregulation of H_2O_2 and collagen I. During this process, the transcription of MMP-1 was pronounced at 24 h (12-fold) and 48h (10-fold) after exposed to DDC in LX-2 co-cultured with C3A-2E1, while the collagen I transcription kept stable, suggesting a post-transcription regulation of collagen I. Accordingly, DDC induced a time-dependent upregulation of MMP-1 both in the supernatant and in LX-2 cells co-cultured with C3A-2E1, while the secretion of collagen I were significantly reduced, suggesting the downregulation of collagen I due to the upregulation of MMP-1. Furthermore, the results of Phospho-MAPK Array Proteome showed that when exposed to DDC the AKT signaling pathway was activated and the ERK1/2 and p38 signaling pathways were inhibited in LX-2 co-cultured with C3A-2E1, and western blot results further confirmed these changes on the signal pathways.
Conclusions: These results suggested that increased expression and secretion of MMP-1 by DDC was responsible for the decrease in collagen I protein produced by the C3A-2E1 co-culture, and the upregulation of MMP-1 by DDC may be associated with the modulation of ERK1/2, p38 and AKT signaling pathways.

PP15-41

Regulation of Inflammation-Driven ECM Remodelling by CD147 and its Interaction with Extracellular Cyclophilins

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Introduction: CD147 is a multifunctional glycoprotein and is involved in inflammatory cell recruitment as the only known receptor for pro-inflammatory extracellular cyclophilins A and B (CyPA). Blocking this interaction has previously been reported to reduce tissue pathology in murine models of asthma, lung inflammation and rheumatoid arthritis. The current study addressed the role of CD147 in the inflammatory response to chronic liver injury and down-stream effects on ECM remodelling in mice of two genetic backgrounds (C57bl/6 and balb/c) treated with CCl_4 for 4 weeks.
Methods: 8 week old C57bl/6 or balb/c mice were treated with CCl_4 (12% in paraffin oil) twice weekly as well as either an anti-CD147 antibody or IgG2a isotype control (HB-189) (100µg administered twice weekly). Injury was assessed by LFTs and histology. Inflammation was assessed by IHC staining of CD45 and, ECM remodelling was assessed by analysis of tissue Hydroxyproline content and net tissue MMP activity. qPCR was used to analyse expression of TNF-α, IFN-γ, TGF-β, α-SMA, Col1a, Col4a, MMP-2, MMP-9 and MMP-13.
Results: After 4 weeks of CCl_4 administration, anti-CD147 intervention in C57bl/6 mice led to a 37% reduction in inflammatory cell aggregates in liver tissue, associated with a decrease in serum ALT and a reduction in Hydroxyproline. In balb/c mice, a 50% reduction in inflammatory cell clusters was also observed as well as reduced serum AST, however this was associated with increased tissue Hydroxyproline and decreased gene expression of TGF-β, α-SMA and MMP-13 and increased IFN-γ.
Conclusion: These studies demonstrate the importance of the novel interaction between CD147 and Cyclophilins in the formation of inflammatory cell clusters, associated with progressive injury. Further, the downstream effects of blocking this interaction are dependent on the genetic background of mice.