1394
15-DEOXY-Δ12,14-PROSTAGLANDIN J2 ATTENUATES THE LIVER FIBROSIS IN CARBON TETRACHLORIDE TREATED MICE

Huanwei Zheng[1], Jidong Jia[1], Liying Li[2]; [1]Liver Center, Beijing Friendship Hospital, Capital Medical University, Beijing, China; [2]Department of Cell Biology, Capital Medical University, Beijing, China

BACKGROUND We have previously showed that the cyclopentenone, 15-Deoxy-Δ12,14-prostaglandin J2 (15-d-PGJ2), exerts potent antifibrogenic properties by inhibiting the proliferation of human myofibroblasts (hMF) and reducing its capacities to synthesize collagen in vitro. Howere, its antifibrogenic effect in vivo remains unknown. In this study, we investigate the antifibrotic effect of 15-d-PGJ2 on carbon tetrachloride (CCl4) induced chronic liver injury in mice. MATERIALS AND METHODS Experimental male C57 mice were randomized into three groups: Normal group, CCl4 group and 15-d-PGJ2 treated group (n=8). In CCl4 group and 15-d-PGJ2 treated group, mice were subject to intraperitoneal injections with 10% CCl4 (1:9 in olive oil) at 10ml/kg twice weekly. Olive oil was given in Normal group. 15-d-PGJ2 (1:1 in DMSO, diluted in saline solution) was intraperitoneally injected at 0.2mg/kg twice weekly in 15-d-PGJ2 treated group. Vehicle for 15-d-PGJ2 (DMSO diluted in saline solution) was administrated to mice in Normal group and CCl4 group. Mice were subject to administration of CCl4 with 15-d-PGJ2 for 4 and 8 weeks. RESULTS Compared to CCl4 groups, the liver fibrosis score, area percentage of collagen and tissue hydroxyproline contents were lower in 15-d-PGJ2 groups; collagen type III mRNA only decreased in week 4, and collagen type I mRNA decreased at week 8; TIMP-1 mRNA decreased in week 8, but it did not decrease in week 4; α-SMA mRNA decreased in either week 4 or week 8. In addition, compared to Normal groups, PPARγ mRNA apparently decreased in CCl4 groups and 15-d-PGJ2 groups, however, remained unchanged between these two groups in week 4 and week 8. No significant differences were seen in the activaty of MMP-2 and MMP-9 between 15-d-PGJ2 groups and CCl4 groups. Immunohistochemistry showed that α-SMA positive and α-SMA/PCNA double-positive cells were remarkedly decreased by 15-d-PGJ2 either week 4 or week 8. Westernblot showed that α-SMA markedly decreased in 15-d-PGJ2 groups. There were no significant differences in necroinflammatory activity score between CCl4 groups and 15-d-PGJ2 groups. CONCLUSIONS 15-d-PGJ2 attenuates the liver fibrosis induced by CCl4 in mice. 15-d-PGJ2 exerts its anti-fibrotic effects by inhibiting the activation and proliferation of HSCs, by which it can reduce collagen type III production at early stage, collagen I and TIMP-1 production at advanced stage, leading the decreasing deposition and degradation of fibrosis. The mechanism underlying this anti-fibrotic effect of 15-d-PGJ2 are PPARγ-independent and not due to its anti-inflammation effects.

Disclosures:

The following people have nothing to disclose: Huanwei Zheng, Jidong Jia, Liying Li

1395
PENTOXIFYLLINE ATTENUATES ESTABLISHED LIVER FIBROSIS AND HEPATIC STELLATE CELL ACTIVATION IN RAT LIVER FIBROSIS

Mehmet R. Mas[1], Gokhan Bilgin[2], Yildirim Karslioglu[3], Bilgin Comert[1], Levent Ozsari[1], Ilker Tasci[1], Nuket Mas[4], Ahmet T. Isik[1], Ozden Uzunalimoglu[5]; [1]Internal Medicine, Gulhane School of Medicine, Ankara, Turkey; [2]Surgery, Gulhane School of Medicine, Ankara, Turkey; [3]Pathology, Gulhane School of Medicine, Ankara, Turkey; [4]Anatomy, Kocatepe University, Afyon, Turkey; [5]Turkish Hepatology Foundation, Ankara, Turkey

Introduction: Liver fibrosis is characterized by overproduction and accumulation of extracellular matrix proteins in the sinusoids. Modulation of hepatic stellate cell (HSC) transdifferentiation may be an effective target to prevent liver fibrosis. Pentoxifylline, a xanthine derivative fosfodiesterase inhibitor with vasodilatory and hemorheologic effects, has immunomodulatory functions. It was reported to alter neutrophil and macrophage functions, and decrease production of reactive oxygen species during inflammatory response. Pentoxifylline has also been shown to inhibit proliferation of skin fibroblasts and HSCs in-vitro, and suppress collagen production by these cells. Though it was reported to prevent fibrous tissue production in-vivo, this action was questioned by some authors. In the present study, the effects of pentoxifylline on established rat liver fibrosis after bile duct ligation (BDL) was studied with reference to activation of HSCs. Materials and Methods: Forty female Spraque Dawley rats underwent BDL. Four out of 36 survived animals were killed after four weeks and establishment of liver fibrosis was confirmed. The remaining 32 rats were randomized into two groups. Group I (n=16) was treated with oral pentoxifylline (50 mg/kg/day) for four weeks. Group II (n=16) did not receive any treatment. After the animals were killed, liver fibrosis, liver tissue hydroxyproline, ductal proliferation and the number of activated HSCs were determined. Results: Serum ALT levels and histological fibrosis scores were lower in group I (365.6±33.2 vs. 406.7±57.7, $p<0.05$, and 44.5±16.7 vs. 67.2 ±11.8, $p<0.003$). On the other hand, tissue hydroxyproline content and the degree ductal proliferation were similar in the two groups (1.71±0.22 vs. 1.88 ±0.09, $p>0.05$, and 3(2,5–4) vs. 4(4–4), $p>0.05$, respectively). alfa-SMA positive HSC counts were significantly lower in the pentoxifylline treated group (15.56±4.22 vs. 29.56±2.56, $p<0.001$). Conclusion: After pentoxifylline treatment, histological parameters of fibrosis improved significantly in correlation with a reduction in the amount of activated HSCs. However, it showed no significant effect on biochemical parameters of liver injury and tissue hydroxyproline content. The results indicate that pentoxifylline may prevent progression of liver fibrosis through inhibiting activation of HSCs even in an established state and continuing injury.

Disclosures:

The following people have nothing to disclose: Mehmet R. Mas, Gokhan Bilgin, Yildirim Karslioglu, Bilgin Comert, Levent Ozsari, Ilker Tasci, Nuket Mas, Ahmet T. Isik, Ozden Uzunalimoglu

1396
ELEVATED SERUM β-GLUCURONIDASE REFLECTS HEPATIC LYSOSOMAL FRAGILITY FOLLOWING TOXIC LIVER INJURY IN RATS

Joseph George[1], Mikihiro Tsutsumi[2]; [1]University of South Carolina School of Medicine, Columbia, SC; [2]Nara Medical University, Kashihara, Japan

The level of serum β-glucuronidase increases in various pathological conditions, including liver disorders. The aim of this

investigation was to study the changes in liver lysosomal membrane stability during experimentally induced hepatic fibrosis that may result in the elevation of serum β-glucuronidase. Liver injury was induced by intraperitoneal injections of N-nitrosodimethylamine (NDMA) in adult male albino rats over 3 weeks. The progression of fibrosis was evaluated histopathologically as well as by monitoring liver collagen content. Lipid peroxides and β-glucuronidase levels were measured in the liver homogenate and subcellular fractions on days 0, 7, 14, and 21 after the start of NDMA administration. Serum β-glucuronidase levels were also determined. A significant increase was observed in β-glucuronidase levels in the serum, liver homogenate, and subcellular fractions, but not in the nuclear fraction on days 7, 14, and 21 after the start of NDMA administration. Lipid peroxides also increased in the liver homogenate and the lysosomal fraction. The measurement of lysosomal membrane stability revealed a maximum lysosomal fragility on day 21 during NDMA induced fibrosis. In vitro studies showed that NDMA has no significant effect on liver lysosomal membrane permeability. The results of this investigation demonstrated that lysosomal fragility increases during NDMA-induced hepatic fibrosis, which could be attributed to increased lipid peroxidation of lysosomal membrane. In this study, we also elucidated the mechanism of increased β-glucuronidase and other lysosomal glycohydrolases in the serum during hepatic fibrosis.

Disclosures:

The following people have nothing to disclose: Joseph George, Mikihiro Tsutsumi

1397
PHOSPHODIESTERASE 4 ISOZYMES PLAY A CRITCAL ROLE IN THE PATHOGENESIS OF CHOLESTATIC LIVER INJURY AND FIBROSIS

Leila Gobejishvili[1], Jingwen Zhang[1], Swati Joshi-Barve[1], Craig J. McClain[1,2], Shirish Barve[1]; [1]Medicine/Gastroenterology, University of Louisville, Louisville, KY; [2]Louisville VAMC, Louisville, KY

Chronic cholestatic liver disease can be created by experimental bile duct obstruction, and it is observed in a variety of clinical settings including primary biliary cirrhosis, extrahepatic biliary obstruction, tumor compression, various drugs and total parenteral nutrition, to name only a few. However, the molecular mechanisms involved in cholestatic liver injury and fibrosis remain largely undetermined. cAMP exhibits hepatoprotective properties including protection against hydrophobic bile salt-induced cytolytic damage. Additionally, cAMP attenuates production of inflammatory and fibrogenic cytokines. The present study was carried out to test the hypothesis that increased expression/activity of cAMP hydrolyzing PDE4 isozymes plays a pathogenic role in the development of hepatic injury and fibrosis during cholestasis. To address this aim we specifically examined the expression/activity profiles of the PDE4 isozymes during cholestasis induced by bile duct ligation in rats. We also examined the effects of selective inhibition of PDE4 isozymes on the development of hepatic injury and fibrosis in this model system. Hepatocyte apoptosis was assessed by the TUNNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling) assay, and liver injury by histopathology and serum alanine aminotransferase (ALT) determinations. Real-time polymerase chain reaction was used to measure mRNA transcripts for markers of hepatic inflammation, hepatic stellate cell (HSC) activation, and fibrosis. Immunohistochemistry for α-smooth muscle actin (SMA) and collagen deposition was performed to identify HSC activation and fibrosis. Notably, a significant induction in the PDE4 expression/activity was observed in conjunction with the development of hepatic injury and fibrosis. Administration of PDE4 specific inhibitor (rolipram) inhibited hepatic PDE4 activity and resulted in a marked attenuation of hepatocyte apoptosis and liver injury as assessed by serum ALT. Moreover, mediators of hepatic inflammation and HSC activation and fibrosis as assessed by TNFα, TGF-β1, SMA and Col1A1mRNA expression and immunohistochemistry were markedly attenuated in BDL animals receiving rolipram. These results strongly support a major pathogenic role for PDE4 isozymes in the development of liver injury, inflammation, and hepatic fibrogenesis during experimental cholestasis and suggest that this pathway could potentially serve as a novel therapeutic target.

Disclosures:

The following people have nothing to disclose: Leila Gobejishvili, Jingwen Zhang, Swati Joshi-Barve, Craig J. McClain, Shirish Barve

1398
THE VALUE OF SERUM RETINOL BINDING PROTEIN AND TRANSFERRIN AS FIBROSIS MARKERS FOR THE PREDICTION OF SEVERITY IN PATIENTS WITH CHRONIC LIVER DISEASE

Jung Hyun Kwon, Si Hyun Bae, Jin Dong Kim, Chan Ran You, Hyun Young Woo, Jeong Won Jang, Chang Wook Kim, Jong Young Choi, Se Hyun Cho, Seung Kew Yoon, Sang Wook Choi; Internal Medicine, College of Medicine, Catholic University, Seoul, South Korea

Background/aims Serum hyaluronic acid (HA) was introduced as a useful marker of hepatic fibrosis. Retinol binding protein (RBP) and transferrin (TF) are known to be correlated with the hepatic biosynthetic capacity. The aim of this study was to evaluate if the serologic markers, HA, RBP and TF could predict the disease severity in patients with chronic liver disease (CLD). **Methods** We analyzed the data of 434 patients (48 control, 125 chronic hepatitis (CH), 178 compensated liver cirrhosis (LC), 83 decompensated LC). The cause of CLD was ranked by the hepatitis B virus (65.0%), alcohol (10.8%), hepatitis C virus (9.7%) and nonalcoholic steato-hepatitis (2.8%). HA was measured by enzyme-linked binding protein assay, RBP and TF by immunonephelometric assay. **Results** The mean age of patients was 54.4 year-old and sex ratio was 290/144 (male/female). In CLD patients, RBP and TF were significantly reduced compared with control subjects and significantly correlated with the severity of CLD in contrast with the HA inversely correlated (control, CH, compensated LC, decompesnated LC; RBP 4.3±1.6, 4.0±2.3, 2.5±1.5, 1.5±0.8 mg/dL, TF 246.1±43.8, 237.4±46.7, 223.3±50.6, 175.4±54.5 mg/dL, HA 49.2±97.4, 68.5±88.0, 215.9±188.8, 512.3±351.4 ng/mL, P<0.000, respectively). Area under receiver operating characteristic curve (ROC) of RBP and HA for the compensated LC were 0.616 and 0.581, respectively and for decompensated LC, were 0.845 and 0.785, respectively. In the patients with CLD, RBP, HA and TF were correlated with serum albumin, platelet count, and prothrombin time and showed the similar area under ROC. Interestingly, there were few biochemical parameters correlated with HA, RBP, and TF in control subjects. HA and RBP were also correlated with the fibrosis score in histology (n=42, P<0.05). After antiviral therapy (n=33, median interval 1026 (158-2495) days), RBP, the level of serum ALT and HBV DNA were only significant improving factors among several biochemical parameters (p<0.05). **Conclusion** Our study suggested that HA, RBP and TF were serologic markers for the prediction of the disease severity in patients with chronic liver disease, not the healthy subjects. Also the RBP may be useful as a surrogate marker of the antiviral effect instead of the liver biopsy.

Disclosures:

The following people have nothing to disclose: Jung Hyun Kwon, Si Hyun Bae, Jin Dong Kim, Chan Ran You, Hyun Young Woo, Jeong Won Jang, Chang Wook Kim, Jong Young Choi, Se Hyun Cho, Seung Kew Yoon, Sang Wook Choi