

The 21st Conference of the Asian Pacific Association for the Study of the Liver

Oral Presentations

17 February, 2011 (Thursday)

Endoscopic Course: Unique Scenarios in Pancreatic obiliary Endoscopy
08:15–11:45

Part I: Endoscopy in Cirrhosis and Portal Hypertension

EC-01

Portal Biliopathy

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The spectrum of abnormalities of the entire biliary tract including intra-hepatic and extrahepatic bile ducts, cystic duct and gall bladder in patients with portal hypertension is called as portal biliopathy. It is also known as portal hypertensive biliopathy, pseudosclerosing cholangitis and portal cavernoma associated cholangiopathy. Most common aetiology is extra-hepatic portal vein obstruction (EHPVO). Other causes include cirrhosis of liver, NCPF and congenital fibrosis. In EHPVO, portal vein is replaced by portal cavernoma and development of choledochal and gall bladder varices. The biliary abnormalities include irregularities in CBD and hepatic ducts, localised saccular dilatations and filling defects suggestive of CBD calculi, etc. Left hepatic duct is most often affected. The incidence of portal biliopathy on ERCP is reported to be in range of 81–100%, but only small percentage of these patients will be symptomatic (5–14%). Biliary strictures develop due to ischemia and vascular bile duct injury or prolonged compression of biliary tract by portal cavernoma. Majority of the patients are asymptomatic. They can present with abdominal pain, recurrent cholangitis and obstructive jaundice. Diagnosis is established by ERCP which also delineates biliary changes and detects if endotherapy is required. MRCP and MR Portography is as accurate as ERCP, in addition it also detects biliary changes, presence of collaterals, shuntable vein, portal cavernoma and helps to differentiate between bile duct varices and CBD stones. Endosonography is a useful addition to the battery of investigations. The natural history of biliopathy is not yet clear. At present there is no consensus on optimal treatment. Asymptomatic patients do not require any treatment. Treatment in symptomatic patients has to be individualised. Treatment options include endoscopic sphincterotomy, stone extraction, and biliary stricture dilatation with or without stenting. Care has to be taken while doing sphincterotomy due to high risk of bleeding due to ampullary and juxtra ampullary varices. Bleeding is managed like any other variceal bleeding. Endoscopic treatment is preferred for CBD stone and dominant strictures. Bilioenteric anastomosis is difficult due to surrounding collaterals. Some authors suggest shunt surgery which may itself improve biliary obstruction, if not then do biliary bypass.

EC-02

Safe Sedation And Monitoring During Endoscopy In Cirrhosis

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Patients with cirrhosis of the liver have a higher sedation risk during endoscopy. Alterations in liver blood flow, portosystemic shunting and drug metabolizing pathways occur which would impact adversely on the pharmacokinetics of sedative medications. Cirrhotic patients are therefore highly sensitive to drugs used for sedation. Midazolam, fentanyl and pethidine are all metabolized in the liver. As such patients with cirrhosis would have decreased metabolism of these drugs and consequent higher plasma level and longer duration of action. Furthermore, many patients with well compensated cirrhosis have subclinical encephalopathy with increased neurosensitivity to sedative drugs. The risk of sedation and analgesia is further compounded and hepatic encephalopathy may be precipitated. Renal impairment, fluid overload, and alcohol and drug abuse are additional problems to consider that may have an effect on the level of sedation, recovery time, and potential toxicity. Cirrhotic patients often undergo gastroscopy for upper gastrointestinal bleeding due to bleeding esophagogastric varices. The risk of encephalopathy is potentiated by the bleeding and blood in the gastrointestinal tract. Owing to the risks involved, there have been recommendations for unsedated endoscopy for patients with cirrhosis. While this may be feasible for quick and uncomplicated diagnostic gastroscopy or even colonoscopy, complicated procedures involving therapy of esophagogastric varices by ligation or sclerotherapy would require some form of sedation. When sedation is administered in these patients, very close monitoring must be carried out. Half or even quarter doses of sedative and analgesic drugs should be given initially and carefully titrated. It should be remembered that the half life of such drugs in cirrhotic patients is prolonged. The principle would be to give as little sedation as required in these patients.

EC-03

Gastric Variceal Bleeding: TIPS

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Amongst the treatment options described for gastric varices, TIPS and variceal obliteration using cyanoacrylate or glue are ones most commonly used. While both therapies are highly effective in controlling gastric variceal bleeding, there is sparse literature comparing

PP15-35

Effects of Magnesium Isoglycyrrhizinate on TGF- β /Smad Signal Expression in Hepatic Stellate Cells in RatsZ.-T. Peng¹, S.-J. Long¹, Y.-X. Liu², J. Li¹, P. Wang¹¹Infectious Diseases, The First Affiliated Hospital of Nanhua University, Hengyang; ²Infectious Diseases, The Third People's Hospital of Shenzhen, Shenzhen, China

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- β_1 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- β_1 , Smad3, Smad7 mRNA.

PP15-36

Long-term Transforming Growth Factor- β 1 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- β 1 (TGF- β 1) is the key regulator in the initiation and progression of liver fibrosis, and we have learned TGF- β 1 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- β 1 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- β 1 treatment, the cells were cultured in 10% FBS-DMEM/F12 medium containing 1ng/ml TGF- β 1 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- β 1 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- β 1 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- β 1. Furthermore, TGF- β 1 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- β 1 exposure.

Conclusion: Long-term TGF- β 1 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- β

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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 (CCl₄) induced hepatic fibrosis mice models were used for in vivo study and hepatic stellate cells (HSCs) line for in vitro study. CCl₄ 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- β 1 (TGF- β 1), α -smooth muscle actin (α -SMA), and fibronectin in explanted liver. Immunoblotting of α -SMA, phosphorylated Smad-2 and Smad-2/3 was performed in HSCs treated with TGF- β 1 and/or rhEPO.

Results: Expressions of TGF- β 1, α -SMA, and fibronectin were increased in CCl₄ injected mice livers, but significantly attenuated by co-treatment with CCl₄ and rhEPO. Co-treatment of rhEPO markedly suppressed fibrosis in Masson's trichrome compared to treatment of only CCl₄. 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- β 1 increased phosphorylated α -SMA, Smad-2 expressions in HSCs, which were decreased by rhEPO co-treatment.

Conclusions: Treatment of rhEPO effectively suppressed fibrosis in CCl₄-induced liver fibrosis mice models. Anti-fibrosis effect of rhEPO could be related to inhibition of TGF- β 1 induced activation of HSCs.

PP15-38

Human MMP-1 Transgene Protects Experimentally Induced Hepatic Fibrosis in MiceJ. George^{1,2}, J. D'Armiento², M. Tsutsumi¹¹Department of Gastroenterology and Hepatology, Kanazawa Medical University, Uchinada, Ishikawa, Japan; ²Division of Molecular Medicine, Department of Medicine, Columbia University, New York, NY, USA

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- β 1 (TGF- β 1) and procollagen-III peptide in wild-type mice. There was marked activation of hepatic stellate cells, deposition of collagen-I 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, α -smooth muscle actin (α -SMA), and TGF- β 1 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

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

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Introduction: Adiponectin 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-parenchymal 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, immunoblot and flow cytometry. NO metabolite concentrations (NO₂/NO₃) 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 (NO₂/NO₃) 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 nonparenchymal 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 diethylthiocarbamate (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 H₂O₂ level and collagen I expression increased in LX-2 co-cultured with C3A-2E1, and DDC inhibited the upregulation of H₂O₂ 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 CCl₄ for 4 weeks.

Methods: 8 week old C57bl/6 or balb/c mice were treated with CCl₄ (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 CCl₄ 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.