Volume 42 Supplement 4 October 2010 ISSN 1590-8658

ELSEVIER

# Digestive and Liver Disease

An International Journal of Gastroenterology and Hepatology

ASSOCIAZIONE ITALIANA PER LO STUDIO DEL FEGATO
A I S F
• 1970 •

**Abstracts of the A.I.S.F. - Italian Association for the Study of the Liver - Monothematic Conference 2010**
**"The Pathologies of the Intra- and Extra-Hepatic Biliary Tree"**
**Rome, October 7th - 8th, 2010**

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**Vol. 42 Supplement 4 (October 2010)**

*Index Medicus (MEDLINE), Current Contents/Clinical Practice,*
*Science Citation Index and EMBASE/Excerpta Medica*
*Sociedad Iberoamericana de Información Científica (SIIC)*

*Associato alla Unione Stampa Periodica Italiana*

## Abstracts of the A.I.S.F. – Italian Association for the Study of the Liver – Monothematic Conference 2010

## "The Pathologies of the Intra- and Extra-Hepatic Biliary Tree"

## Rome, October 7th – 8th, 2010



**Abstract Legend**

**OC:** Oral Communications
**P:** Posters

Ductular reactive cells and HPCs number were determined by computer-assisted morphometric analysis of panCK and CK19 immunolabeling. Biliary committed progenitor cells were identified by SOX-9 immunolabeling. An *in vitro* system to assess tubule formation was developed by culturing a mice progenitor cell line (BMOL) into a Matrigel sandwich. Tubule length and split tips were counted.
**Results:** In both Notch-2-cko and RBP-Jκ-cko mice a significant degree of biliary immaturity and paucity was present and persisted at post-natal days P8, P28, P56. Bile duct paucity was more severe in the RBP-Jκ-cko mice. Liver repair was dramatically altered both after DDC and ANIT treatment. Notch-2-cko mice showed abundant cytokeratin-positive structures that never formed mature ducts; in RBP-Jκ-cko mice, ductular reaction was virtually absent and parenchymal necrosis was present. In Notch-2-cko mice SOX-9-positive HPCs were increased, but were virtually absent in RBP-Jκ-cko mice. Expression of the Notch ligand Jagged-1 was increased in Notch-2-cko mouse livers, suggesting a compensatory stimulation of other Notch receptors to generate HPCs. GSI administration to DDC-treated WT mice significantly reduced ductular reaction and HPC. BMOL formed a network of interconnecting tubules which length and branches were significantly reduced by treatment with GSI, and by siRNA silencing of Notch2 and Jagged1.
**Conclusions:** Notch contributes to liver repair by regulating tubule formation and the generation of biliary-committed precursors. Lack of Notch-2 receptor prevents biliary tubule formation, both in vivo and in vitro. Lack of RBP-Jk, which causes a more severe defect of both Notch1 and Notch2 signaling, inhibits the generation of biliary-committed precursors in addition to tubule formation.

## OC9

### Isolation, culturing and differentiation of multipotent stem cells from human foetal biliary tree

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**Background:** The biliary tree, liver and pancreas share a common embryological origin from the elongation of primitive foregut. We have demonstrated the presence of multipotent endodermal stem cells (MPS) in the adult human extra-hepatic biliary tree, located at the bottom of peribiliary glands, capable to differentiate *in vitro* into hepatocytes, cholangiocytes and pancreatic islet cells.
**Aims:** The aims of study were to isolate, characterize and culture stem cells/progenitors from the biliary tree of fetal livers.
**Materials and method:** Foetal livers were provided from fetuses at 18–22 weeks gestational age, obtained from elective terminations of pregnancy. Intra-hepatic (IHBT) and extra-hepatic biliary tree (EHBT) were digested with Collagenase and freshly isolated cells were plated onto plastic in Kubota's Medium (KM) added with different growth factors to favor a selective differentiation of MPS into hepatocyets, cholangiocytes and pancreatic cells. Phenotype of biliary tree fragment and of MPS was investigated by immunofluorescence (IF).
**Results:** IF of biliary tree showed that the ductal plate and forming interlobular ducts expressing EPCAM, NCAM, CK7, CK19, coexist with formed large bile duct expressing EPCAM, NCAM and CK19. An average of 16 million cells were isolated from IHBS and 5 millions cells from EHBS. Cells were expanded for more than 45 days in KM and showed a similar *in vitro* phenotype. MPS cells were then effectively induced to differentiate into mature cholangiocytes, hepatocytes and pancreatic islets by specific media. Media added with HGF, EGF, oncostatin M induce in 7–10 days the appearance of hepatocytes CK18 (+) and albumin (+). Media added with HGF, EGF and calcium induce MPS to differentiate in cells expressing CK19 and secretin-receptors. Media added with exendin-4, B27, calcium, ciclopamine, ascorbic acid induced MPS to differentiate into cells expressing insulin and C-Peptide.
In **conclusion**, we have isolated MPS from fetal biliary tree and induced their *in vitro* differentiation into mature hepatocytes, cholangiocytes and pancreatic cells secreting insulin. Therefore, fetal biliary tree represents a source of MPS that could be considered for cell therapy of liver diseases.

## OC10

### Combination therapy ezetimibe/simvastatin decreases the supersaturation in gallbladder bile and prevents cholesterol gallstones formation

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**Background and aims:** Cholesterol cholelithiasis is one of the most prevalent and most costly digestive diseases in developed countries and its incidence has increased markedly to the adoption of hyperlipidic dietary. Animal experiments showed that high efficiency of intestinal cholesterol absorption contributes to gallstone formation, beside increased viscosity, supersaturation of cholesterol and impaired motility of the gallbladder; ezetimibe is the first lipid-lowering drug that inhibits intestinal uptake of dietary and biliary cholesterol without affecting the absorption of fat-soluble nutrients reducing the small intestinal enterocyte uptake and absorption of cholesterol by binding to Niemann-Pick C1 Like 1 (NPC1L1), which keeps cholesterol in the intestinal lumen for excretion. Simvastatin is an inhibitor of liver 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCoA) and therefore reduces the liver cholesterol synthesis. We explored whether the overall effects of ezetimibe/simvastatin co-administration could reduce biliary cholesterol content, viscosity and prevent gallstones in human beings and promote gallstone dissolution.
**Patients and methods:** 126 patients (60 males, 66 females, age 49±6) were enrolled showing hypercholesterolemia (serum mean value 283±25.4 mg/dL) and baseline ultrasonographic signs of cholesterol supersaturation in gallbladder bile. Patients were randomized 1:1 into two groups: in group A 63 patients followed a diet low in cholesterol, in group B 63 patients followed a diet low in cholesterol and the co-administration of ezetimibe 10 mg plus simvastatin 20 mg/day for 6 months. After 6 months of therapy in all patients a second ultrasonography was performed to assess the bile appearance in the gallbladder.
**Results:** In group A 10/63 patients (15.8%) showed a reduction in viscosity and supersaturation of cholesterol in gallbladder bile; in group B (diet low in cholesterol with co-administration ezetimibe/simvastatin) 53/63 patients (84.2%) showed a reduction in viscosity and supersaturation of cholesterol in gallbladder bile ($p<0.001$). No adverse events occurred in the group of patients treated with ezetimibe and simvastatin.
**Conclusions:** The data seem to show that co-administration ezetimibe/simvastatin can effectively reduce the biliary cholesterol saturation and delay crystallization. Co-administration ezetimibe and simvastatin is a novel approach to reduce gallbladder bile cholesterol, mud, debris, sand or cholesterol spheroids content (bile desaturation action) and a promising strategy for preventing or treating cholesterol gallstones by inhibiting intestinal cholesterol absorption and reducing liver cholesterol synthesis. Clinical trials will be needed to support these results.

## OC11

### Bilary excretion of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine during ischemia/reperfusion injury

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**Background and aims:** Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) synthase enzyme, whereas symmetric dimethylarginine (SDMA) is not biologically active. The liver represents